# Chapter 7

## Tract Based Spatial Statistics on Diffusion Tensor Imaging in Systemic Lupus Erythematosus Reveals Localized Involvement of White Matter Tracts.

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Arthritis Rheum 2010 Aug 18. [Epub ahead of print]

## ABSTRACT

### Purpose

The aim of this study is to determine whether there are differences between SLE patients and healthy controls in white matter integrity, using tract based spatial statistics (TBSS) on diffusion tensor imaging (DTI) data.

### Methods

Twelve SLE patients (age 42; range 15-61), diagnosed according to the American College of Rheumatology (ACR) revised 1982 criteria for SLE, and 28 healthy controls (age 46; range 21-61) were included in this study. MRI was performed on a 3.0-T scanner. Fractional anisotropy (FA) maps were calculated for each patient. To compare FA maps TBSS was used. TBSS projects the FA data into a common space through the use of an initial approximate non-linear registration followed by projection onto an alignment invariant tract representation (mean FA skeleton). The cluster results were corrected for multiple comparisons across space and a threshold of significance of 0.05 was used.

### Results

The white matter of tracts in the inferior fronto-occipital fasciculus, the fasciculus uncinatus as well as the fornix, the posterior limb of the internal capsule (corticospinal tract), and the anterior limb of the internal capsule (anterior thalamic radiation) showed reduced integrity.

### Conclusion

The integrity of white matter tracts in areas around limbic structures and in the internal capsule was reduced in this preliminary study. Larger studies could improve our understanding of the pathomechanisms behind the reduced white matter tract integrity in SLE.

## INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease. Despite the fact that up to 75% of SLE patients develop neuropsychiatric symptoms, the exact origin of these symptoms is still largely unknown. Abnormalities on MRI, such as white matter hyperintensities and infarcts, are a common finding in neuropsychiatric SLE (NPSLE). (1) However, conventional MRI often fails to provide an explanation for neuropsychiatric symptoms in SLE patients, causing a remarkable clinico-radiological paradox. (2)

Using quantitative MRI techniques, abnormalities can be observed in cerebral gray and white matter that correlate with clinical symptoms in NPSLE patients with unremarkable findings on conventional MRI. (3;4) Furthermore, it has been demonstrated that these cerebral quantitative abnormalities not only occur in NPSLE patients, but also in SLE patients not fulfilling the ACR criteria for NPSLE, suggesting that brain involvement is more widespread among SLE patients than formerly believed. (5;6) Using quantitative MRI techniques, it has been demonstrated that in SLE patients gray matter changes that are invisible on conventional MRI occur in specific locations in the brain. (7;8) A recent MRI study reported abnormalities in the amygdala in human SLE patients, which is in line with the findings in a murine study showing direct antibody-mediated damage to specific limbic brain structures because of local disturbance of the blood-brain barrier. (6) Several studies have shown white matter damage in NPSLE patients. Conventional MRI often shows non specific white matter hyperintensities. (9-13) Quantitative techniques have shown that the white matter shows quantitative abnormalities compared to healthy controls. (4;14-16) However these studies compared the white matter as a whole or specified regions of interest. So far it remains unknown whether white matter damage also has sites of predilection in NPSLE patients.

Diffusion weighted imaging (DWI) is an MRI technique that permits measuring Brownian motion of protons in the brain. (17) Diffusion tensor imaging (DTI), a refined DWI technique, allows assessment of the preferential direction of Brownian motion, which reflects the microscopic architecture of the brain's white matter. Furthermore, DTI permits assessing disease-related changes of white matter integrity in a quantitative fashion. Recently, a technique has been introduced that permits voxelwise statistical analysis of DTI data using tract based spatial statistics (TBSS), which permits robust assessment of local differences in white matter integrity between groups. (18)

The aim of this study is to assess, using TBSS, the presence and location of white matter damage in NPSLE patients without findings on conventional MRI that explain their symptoms. (18) Based on earlier observations of gray matter damage in the mesial temporal lobe, we hypothesized that white matter changes secondary to such gray matter damage (throughWallerian degeneration) preferentially affects the white matter tracts in areas around gray matter structures of the limbic system.

## METHODS

### Subjects

All SLE patients were recruited from our tertiary referral outpatient clinic for SLE patients with neuropsychiatric complaints. Twelve SLE patients, diagnosed according to the ACR revised 1982 criteria for SLE, and 28 healthy controls were included in this study. Subjects with obvious infarction, numerous white matter hyperintensities or other macroscopic damage on conventional MRI were excluded from the analysis. The Institutional Review Board approved the study and written informed consent was obtained from all subjects. All patients were exeamined by a rheumatologist. Special care was taken to exclude patients with any possible secondary NP complaints, due to drug effects, alcohol or drug abuse, or concurrent disease. Controls were screened for mediaction use and control subjects with medication were excluded from the study.

### Data acquisition

Diffusion tensor imaging (DTI) was performed on a 3.0-T Philips Achieva MRI system (Philips Medical Systems, Best, The Netherlands) using single-shot echo-planar imaging (EPI) in combination with an eight channel SENSE head coil used for radiofrequency reception of the nuclear magnetic resonance signals. Parameters for DTI acquisition were as follows: TR = 6269 ms, TE = 48 ms, flip angle = 90°, *b* factor = 800 s/mm², voxel dimensions = 2.00 mm × 2.04 mm × 3.60 mm, FOV = 224, number of slices = 40, slice gap = 0 mm. DTI images were acquired in 6 directions together with a baseline image having no diffusion weighting. Total scan time was approximately 2 minutes for the acquisition of one diffusion weighted data set.

### Data preprocessing

All data were processed using FSL (FMRIB Software Library, FMRIB Center, Oxford). (19) First, each data set was corrected for stretches and shears induced by eddy currents in the gradient coils and simple head motions by using an affine transformation of each diffusion weighted image to the reference volume without diffusion weighting. Next, non-brain matter was removed from the images using BET (Brain Extraction Tool). (20) Finally, a diffusion tensor model was fitted on the data to determine the level of anisotropy for each voxel independently by calculating the tensor eigen values using FDT (FMRIB's Diffusion Toolbox) describing the diffusion strength in the primary, secondary and tertiary diffusion directions. The fractional anisotropy (FA; values between 0 = isotropic and 1 = anisotropic), a quantification of how strongly directional the local tract structure in a voxel is, was then calculated and plotted in a single FA map for each subject (figure 1).

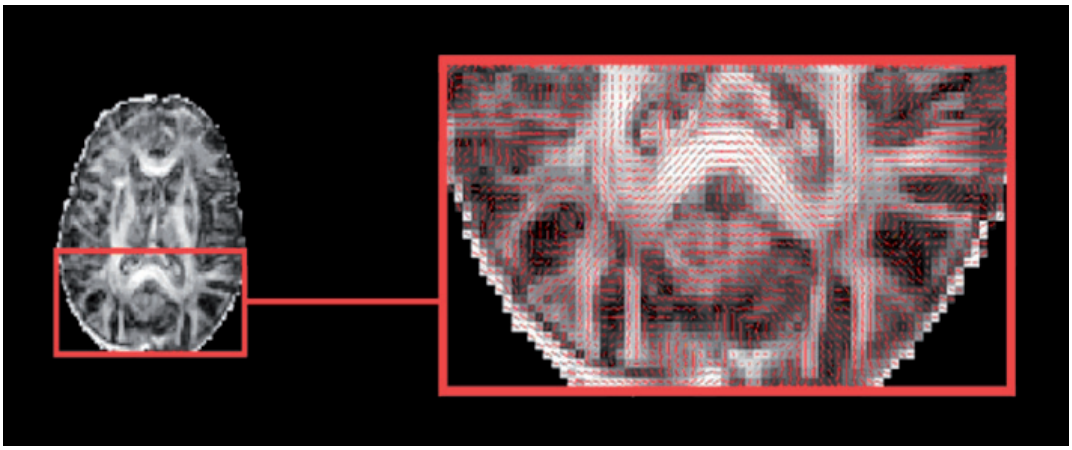

**Figure. 1.** Example output of diffusion tensor fitting on the data. A representative single subject FA map is shown on the left. On the right, an enlargement is shown with the principal eigenvector (i.e. principal diffusion direction) projected as lines onto the FA map per voxel.

### Alignment and Statistics

To allow voxelwise analysis of FA data across subjects, individual FA maps need to be aligned. Application of standard registration algorithms however leads to insufficient overlap between subjects' FA data, causing invalid interpretation of subsequent voxelwise analysis. TBSS (Tract-Based Spatial Statistics; part of FSL) was used to overcome this problem. (18) First, this tool aligns every FA image to every other one, identifies the "most representative" one and uses this as the target image. Next, this target image is affine-aligned to MNI-152 standard space. All other images are then transformed into MNI-152 space by combining the nonlinear transform to the target FA image with the affine transform from that target to MNI-152 space. This results in a standard space version of every subject's FA image. From these new images a mean FA image is calculated to create a mean alignment-invariant tract representation, i.e. the mean FA skeleton, which represents the centers of all tracts common to the group (figure 2). Each subject's aligned

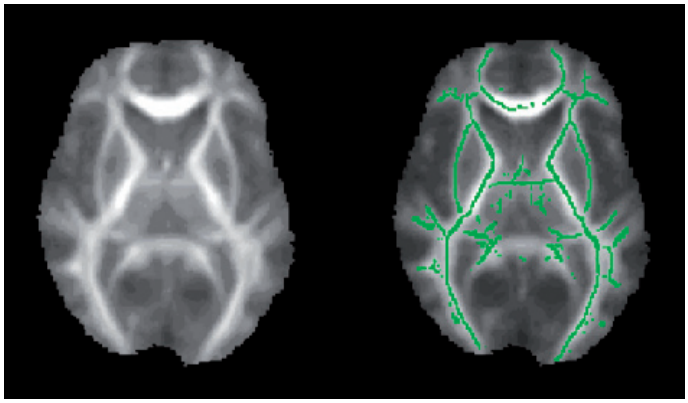

**Figure. 2** The mean FA image (left) and the mean FA skeleton in green (thresholded at an FA value of 0.3) projected onto mean FA image (right).

FA data is then projected onto this skeleton and the resulting data is fed into voxelwise statistics, applying a control-patient unpaired *t* test. Inference was carried out using cluster-size thresholding, with clusters initially defined by $t > 3$. The null distribution of the cluster-size statistic was built up over 5000 permutations of group membership (FSL Randomise tool), with the maximum size (across space) recorded at each permutation. The 95th percentile of this distribution was then used as the cluster-size threshold, i.e., the clusters were thresholded at a level of $P < 0.05$, which is fully corrected for multiple comparisons across space. (18)

## RESULTS

The average age of the patients was 42 (range 15- 61) and 46 years (range 21-61) for controls. Seven of the SLE 12 patients suffered from one or more of the Neuropsychiatric Syndromes as described by the ACR: headache (2), mononeuropathy (1), cranial neuropathy (1), polyneuropathy (1), cerebrovascular disease (1), cognitive disorder (3) and psychosis (1). Average SLE disease duration was 5.6 years (range 0-22) and average duration of neuropsychiatric syndromes was 1.4 years (range 0-10) in seven patients. Five of the 12 patients used prednisone varying in dosage from 10mg to 60mg, one patient received low dose methotrexate, one patient used azathioprine (dosage 50mg), two patients used ascal, five patients used hydrochoroquine (200-400mg), and two patients used non steroidal anti-inflammatory drugs (NSAID's) on a daily basis; other drugs that were used comprised antiepileptic, anti-hypertensive, anxiolytic and anti-depressive drugs.

TBSS results of the comparison of the white matter skeleton of patients and controls are shown in figure 3. There is reduction of white matter integrity, reflected by a reduction of FA values of the white matter skeleton, in several areas in the brain of SLE patients. The integrity of the subcortical white matter tracts of the parietal and frontal lobe is relatively preserved, whereas the frontobasal and temporal regions seem to be predominantly involved. Figure 4 demonstrates that significant differences in white matter tract integrity between SLE patients and healthy controls were mostly found in frontobasal and temporal white matter tracts, including the inferior fronto-occipital fasciculus, the fasciculus uncinatus as well as the fornix, the posterior limb of the internal capsule (corticospinal tract), and the anterior limb of the internal capsule (anterior thalamic radiation). White matter tracts of occipital, parietal and (posterior) frontal lobes did not differ between SLE patients and healthy controls. Areas where FA values were significantly higher for patients than for controls were not found.

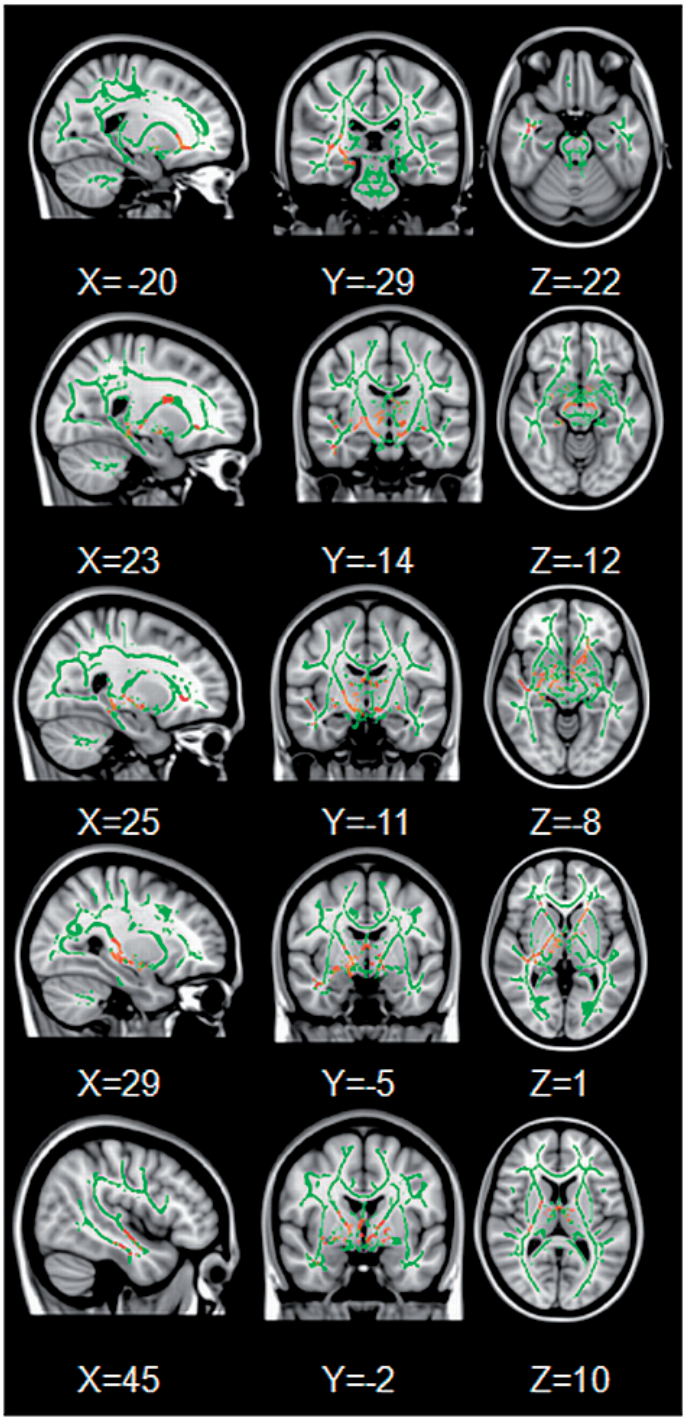


**Figure. 3.** TBSS analysis showing the mean FA skeleton in green (thresholded at an FA value of 0.3) and significant group differences in red to yellow. Mean FA is shown as background. Saggital views are from left to right, coronal from posterior to anterior and axial from ventral to dorsal. Red to yellow indicates the level of significance. These are the areas where significant lower FA values were present in the patients compared to the control group. Areas where FA values were significantly higher for patients than for controls were not found.

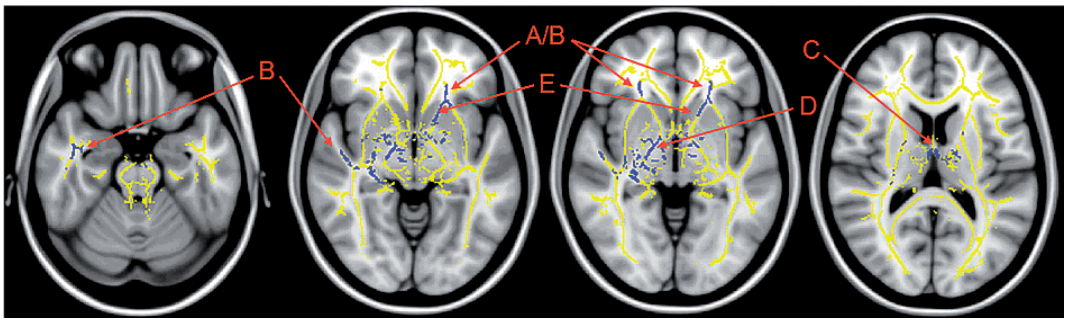


**Figure 4.** Significant differences in white matter tract integrity between SLE patients and healthy controls were mostly found in frontobasal and temporal white matter tracts, including the inferior fronto-occipital fasciculus (A), the fasciculus uncinatus (B) as well as the fornix (C), the posterior limb of the internal capsule (corticospinal tract) (D), and the anterior limb of the internal capsule (anterior thalamic radiation) (E). White matter tracts of occipital, parietal and (posterior) frontal lobes did not differ between SLE patients and healthy controls.

## DISCUSSION

Our results show reduced FA values in the frontobasal and temporal white matter tracts, including the inferior fronto-occipital fasciculus, the fasciculus uncinatus as well as the fornix, the posterior limb of the internal capsule (corticospinal tract), and the anterior limb of the internal capsule (anterior thalamic radiation) in SLE patients as compared to healthy controls. The white matter tracts of occipital, parietal and (posterior) frontal lobes were not significantly different between SLE patients and healthy controls

DWI can be used to measure diffusivity in the brain, providing signal proportional to the molecular diffusion of water molecules based on Brownian motion. (17) Average diffusion coefficient (ADC) maps provide information on the microstructure of tissue and can be very useful in the detection of disease. (21) Previously, volumetric DWI has been used in NPSLE patients to provide quantitative measures of integrity of the whole brain. (22) Such measures comprised mean ADC values of the whole brain volume and descriptive parameters of ADC histograms of the whole brain, such as peak height. Using ADC histograms of the whole brain, Bosma et al found changes in NPSLE patients without relevant changes on conventional MRI that correlated with clinical symptoms. (23) However, the method used in that study did not permit assessing which parts of the brain were responsible for the observed changes in ADC histograms of the whole brain. Recently, in an effort to reproduce an observation from a murine model in patients, ADC measurements were performed locally in the gray matter of the mesial temporal lobe in NPSLE patients with antibodies directed against the NMDA receptor.(6)

DTI is a DWI technique that permits assessing the preferential direction of proton diffusivity. Fractional anisotropy (FA) is a quantitative DTI measure reflecting the degree of directionality of diffusion in a given voxel or region of interest. Areas with coherent

diffusion directions, such as in highly structured tissues like white matter tracts, have higher FA values than areas where the direction of diffusion is less coherent, such as in the cerebrospinal fluid where protons do not experience physical barriers. Furthermore, FA values of white matter may change as a result of pathological processes that affect white matter integrity. (24;25) FA measurements have proven a) to be more sensitive to the presence of disease in brain tissue than conventional MRI and b) to reflect brain tissue integrity in a quantitative fashion. (25) Using DTI, Hughes et al reported differences in thalamus, corpus callosum, parietal and frontal white matter in a group of eight patients, of which seven showed morphological or ischemic abnormalities on the conventional MR sequences compared to healthy controls. (15) Furthermore, Zhang et al localized DTI differences in the corpus callosum, frontal lobe, the anterior and the posterior internal capsule between 14 patients with normal appearing conventional MRI and healthy controls. (16) However, both these studies used region of interest analysis. TBSS permits voxelwise statistical analysis of all DTI data providing robust assessment of local differences in white matter integrity between groups. TBSS projects the FA data into a common space that is not dependent on perfect non-linear registration. This is achieved through the use of an initial approximate non-linear registration, followed by projection onto an alignment invariant tract representation (the mean FA skeleton). In addition, this approach does not require any spatial smoothing, With this approach TBSS circumvents some of the methodological problems of voxel based morphometry (VBM) on FA data. (18;25;26)

Damage caused by antibodies directed against neuronal receptors would be expected to be located at the site of the highest concentration of these neuronal receptors, i.e. in the gray matter. (27) However, our results show localized decreased integrity of the white matter tracts of the inferior fronto-occipital fasciculus, the fasciculus uncinatus as well as the fornix, the posterior limb of the internal capsule (corticospinal tract), and the anterior limb of the internal capsule (anterior thalamic radiation) in SLE patients [Figure 4]. They suggest that the quantitative changes in cerebral parenchyma of SLE patients not visible on conventional MRI are not limited to neurons in the gray matter. (3;4) Several pathogenic mechanisms can be considered. First, besides the internal capsule, the fasciculus uncinatus, fornix and the inferior frontal fasciculus between the limbic system and the limbic association cortex are located around gray matter structures of the limbic system. The involvement of these white matter could be the reflection of axonal damage through Wallerian degeneration caused by damage in the gray matter. Second, involvement of the white matter could be caused directly through subtle noxious influences in NPSLE, such as repeated episodes of acute inflammation in small vessels. This could cause priming or activation of the wall of these small vessels by complement and/or anti-endothelial antibodies. Priming or activation of the vessel wall could subsequently lead to vasculopathy and microinfracts or subtle hypoperfusion in small vessels of the brain, causing subtle

abnormalities not visible on conventional MRI but measurable with DTI. (28) Thirdly, white matter damage could be due to antibodies that have not yet been characterized directed directly against myelin, leading to direct white matter damage through a pervasive attack on axonal myelin sheaths or the oligodendrocytes that they derive from. Finally, reduced integrity of the white matter tracts could be due to a combination of these mechanisms.

Due to strict selection on the basis of conventional MRI, our study comprised a relatively small group of SLE patients: some not fulfilling the ACR criteria for NPSLE, and among those that fulfilled the NPSLE criteria, clinical symptoms varied from pheripheral involment like polyneuropathy to cognitive disorder or psychosis. Furthermore, SLE is a heterogeneous disease and the brain is likely to be affected by different pathomechanisms. In addition, some of the patients were using medication that could have an influence on quantitative MRI values, because of the small size of the study population this is not easily corrected for. Indeed, larger studies are needed to better discern the effects of medication and the effects of antibodies on the brain. Despite these observations and the small patient group we found significant and consistent differences between our patients and healthy controls. In order to improve our understanding of the pathomechanisms responsible for the changes we observed, TBSS analysis has to be performed between more homogeneous groups of patients that are well documented with more extensive laboratory analysis.

In conclusion, our results show reduced FA values in the frontobasal and temporal white matter tracts, including the inferior fronto-occipital fasciculus, the fasciculus uncinatus as well as the fornix, the posterior limb of the internal capsule (corticospinal tract), and the anterior limb of the internal capsule (anterior thalamic radiation) in SLE patients as compared to healthy controls. Larger studies with a more extensive comparison of TBSS analysis with other imaging parameters, clinical and laboratory findings could improve our understanding of the pathomechanisms behind the reduction of the white matter tract integrity in SLE.

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