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Author: Ercan, Ayşe Ece

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Glial and axonal changes in systemic lupus erythematosus measured with diffusion of intracellular metabolites

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A.E. Ercan, C. Magro-Checa, R. Valabregue, F. Branzoli, E.T. Wood, G.M. Steup-Beekman, A. Webb, T. Huizinga, M.A. van Buchem and I. Ronen, "Glial and Axonal Changes in Systemic Lupus Erythematosus Measured With Diffusion of Intracellular Metabolites" *submitted to Brain* (2015).

Abstract

Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disease with multi-organ involvement. Central nervous system involvement in SLE is common and results in several neurological and psychiatric (NP) symptoms that are poorly linked to standard MRI outcome. MRI methods sensitive to tissue microstructural changes, such as diffusion tensor imaging (DTI) and magnetization transfer imaging (MTI), show some correlation with NPSLE symptoms. Histological examination of NPSLE brains reveals presence of cerebral edema, loss of neurons and myelinated axons, microglial proliferation and reactive astrocytosis, microinfarcts and diffuse ischemic changes, all of which can affect both DTI and MTI in a non-specific manner. Here we investigated the underlying cell-type specific microstructural alterations in the brain of SLE patients with and without a history of CNS involvement. We did so combining DTI with diffusion-weighted magnetic resonance spectroscopy (DW-MRS), a powerful tool capable of characterizing cell-specific cytomorphological changes based on diffusion of intracellular metabolites. We used a 7 tesla MRI scanner to acquire T_1 -weighted images, diffusion tensor imaging (DTI) datasets, and single volume DW-MRS data from the anterior body of the corpus callosum of 13 SLE patients with past neuropsychiatric SLE (NPSLE), 16 SLE patients without past NPSLE, and 19 healthy controls. Group comparisons were made between SLE patients with/without past NPSLE and healthy controls on DTI metrics and on diffusion coefficients of three brain metabolites: the exclusively neuronal/axonal N-Acetylaspartate (NAA), and the predominantly glial creatine + phosphocreatine (tCr) and choline compounds (tCho). In SLE patients with past NPSLE, significantly higher DTI mean and radial diffusivities were accompanied by a significantly higher intracellular diffusion of tCr ($0.20 \pm 0.03 \mu\text{m}^2/\text{s}$, $p\text{-value} < 0.01$) and tCho ($0.14 \pm 0.03 \mu\text{m}^2/\text{s}$, $p\text{-value} < 0.05$) compared to healthy controls ($0.17 \pm 0.02 \mu\text{m}^2/\text{s}$, $0.12 \pm 0.02 \mu\text{m}^2/\text{s}$, respectively). tNAA, tCr and tCho diffusion values from all SLE patients correlated positively with SLE disease activity index score ($p\text{-value} < 0.05$, $p\text{-value} = 0.02$, $p\text{-value} < 0.05$, respectively). Our results indicate that intracellular alterations, and in particular changes in glia, as evidenced by increase in the average diffusivities of tCho and tCr, significantly correlate with SLE activity. The higher diffusivity of tCr and tCho in NPSLE patients, as well as the positive correlation of these diffusivities with the SLE disease activity index are in line with cytomorphological changes in reactive glia, suggesting that the diffusivities of choline compounds and of total creatine are potentially unique markers for glial reactivity in response to inflammation.

6.1 Introduction

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) is a female predominant autoimmune disease that affects multiple organs [1]. Central nervous system involvement in SLE is common and results in several neurological and psychiatric symptoms. These symptoms are poorly characterized by standard magnetic resonance imaging (MRI), which appears normal in about 50% of neuropsychiatric systemic lupus erythematosus (NPSLE) patients. Focal lesions and vascular infarcts, visible on MRI of NPSLE patients, are non-specific and often do not correlate with clinical outcome and with symptom severity [2].

MRI methods sensitive to tissue microstructural changes, such as diffusion tensor imaging (DTI) and magnetization transfer imaging (MTI), show diffuse white matter changes that correlate with the clinical status of NPSLE patients [3–7]. Histological examination of NPSLE brains has revealed the presence of cerebral edema, loss of neurons and myelinated axons, microglial proliferation and reactive astrogliosis, microinfarcts and diffuse ischemic changes, all of which can affect the image contrast in DTI, and MTI [8]. Therefore, although clinically informative, due to their lack of specificity, these imaging modalities provide limited insight into the microstructural deficit in NPSLE.

Magnetic resonance spectroscopy (MRS) reports on concentrations of cell-specific metabolites, and MRS studies have shown differences in the concentrations (relative to total creatine) of several brain metabolites, including significantly lower N-Acetylaspartate (NAA) and significantly higher choline and myo-inositol levels in SLE and NPSLE patients compared to healthy controls (HC) [9, 10]. In addition, one study reported significantly lower NAA in SLE patients with high disease activity compared to those with low disease activity [11]. Although MRS provides cell-type specific information, it does not provide any structural information.

Diffusion-weighted magnetic resonance spectroscopy (DW-MRS) combines the cell-type specificity of MRS with the microstructural sensitivity of diffusion-weighted imaging (DWI), and allows studying cell- and compartment-specific properties of tissue microstructure by probing the diffusion of intracellular brain metabolites [12, 12, 13]. Of these metabolites, N-acetylaspartate typically co-measured with N-acetylaspartylglutamate (NAAG) ($\text{NAA} + \text{NAAG} = \text{tNAA}$) resides almost exclusively in neurons/axons; creatine and phosphocreatine ($\text{Cr} + \text{PCr} = \text{tCr}$), pivotal in aerobic cell energetics, are found in all neural cells, but their astrocytic concentration is twice their neuronal one, and soluble choline-containing compounds (tCho) are predominantly glial, with a glial/neuronal concentration ratio of 3:1 [14, 15]. The diffusion properties of these metabolites are strongly dictated by the structural and physiological features of their respective intracellular space, and thus provide a unique in-vivo probe for pathology affecting intracellular structures, such as ischemia [16, 16], tumors [17, 18], and axonopathy in multiple sclerosis [19], as well as making accurate vivo cell-specific characterization of tissue microstructure possible [13, 20].

In this study we utilize for the first time the sensitivity of DW-MRS to selectively report on axonal and glial microstructure (a) to investigate the underlying microstructural alterations in a normal appearing portion of the corpus callosum in the brain of SLE patients with and without history of NPSLE and (b) to assess the relationship between DW-MRS indices and SLE activity in the patient population in this study. These studies were performed at ultrahigh field (7 Tesla) in order to obtain the sensitivity required for

robust DW-MRS measurements.

6.2 Materials and Methods

6.2.1 Human subjects

A total of twenty-nine SLE patients (one male, twenty-eight females, age: 43 ± 10 years) and nineteen age and sex matched healthy volunteers (one male, eighteen females, age: 41 ± 11 years) were included in the study. The study adhered to the Helsinki Declaration and was approved by the institutional review board of our institution. Written informed consent was obtained from all subjects prior to the study. Of the twenty-nine SLE patients, thirteen had a history of NPSLE and sixteen had no history. For convenience, patients with past NPSLE incidence are referred to as “NPSLE patients” throughout the text. All SLE patients were diagnosed according to the 1982 revised American College of Rheumatology criteria [21, 22]. All NPSLE patients were diagnosed at the Leiden NPSLE-clinic after a standardized multidisciplinary medical examination [23]. NP diagnoses were classified according to the 1999 American College of Rheumatology case definitions for NPSLE syndromes [24]. The NP syndromes in our NPSLE cohort included cerebrovascular disease (5 patients), seizures (3 patients), cognitive disorder (3 patients), movement disorder (1 patient), headache (2 patients), acute confusional state (3 patients), psychosis (1 patient), transverse myelitis (1 patient), polyneuropathy (1 patient), anxiety (1 patient), and radiculopathy (1 patient). In order to categorize the patients according to SLE disease activity, we calculated the systemic lupus erythematosus disease activity index 2000 (SLEDAI-2K) for each patient [25]. Permanent and irreversible damage due to SLE was assessed with the systemic lupus international collaborating clinics (SLICC)/American College of Rheumatology damage index (SDI) [25, 26]. SLE patients with a SLEDAI-2K ≥ 8 were considered to have high SLE activity and were categorized as SLE-active [11], while the remaining SLE patients were categorized as SLE-inactive. The demographics of the study and the clinical characteristics of the SLE patients are shown in Table 6.1.

6.2.2 Data Acquisition

All subjects were scanned on a 7 Tesla Philips Achieva MRI scanner (Philips Healthcare, Best, The Netherlands) equipped with a 32-channel receive head coil (Nova Medical Inc., Wilmington, MA, USA). The scan protocol consisted of a short survey scan and a sensitivity encoding reference scan followed by (a) sagittal 3D T_1 -weighted images (FOV: $246 \times 246 \times 174$ mm³, resolution: $0.85 \times 0.85 \times 1$ mm³, TR/TE: 4.00 / 1.84 ms, total scan time: 1.59 min); (b) axial multislice diffusion tensor images (FOV: $224 \times 224 \times 150$ mm³, resolution: $1.75 \times 1.75 \times 2.20$ mm³, TR/TE: 10000/65 ms, fifteen diffusion weighting directions with $b = 1000$ s/mm², total scan time: 3 min) and (c) single-volume diffusion-weighted spectroscopy scans (detailed protocol below).

6.2.2.1 DW-MRS Protocol

The DW-MRS sequence was based on the PRESS (Point Resolved Spectroscopy) sequence with bipolar diffusion-weighting gradients added on both sides of the 180° pulses. A 3 cc volume of interest (VOI) (25 (AP) $\times$ 15 (RL) $\times$ 8 (FH) mm³) was positioned on the anterior body of the corpus callosum as shown in Figure 6.1. The diffusion-weighting gradients were applied in two directions: a right-left direction in the VOI frame, mostly parallel to the direction of the callosal fibers (direction [1, 0, 0]), and a direction mostly

TABLE 6.1: Patient characteristics.

		NPSLE patients (n=13)	SLE patients (n=16)
Patient characteristics	Age (years)	43 ± 8	42 ± 11
	SLE disease duration (years)	12 ± 9	8 ± 5
	SLEDAI - 2K	7 ± 6	3 ± 2
	SDI	2 ± 2	1 ± 1
Auto-antibodies	Antiphospholipid syndrome	3 (23%)	1 (6%)
	Incidence of past NPSLE	13 (100%)	0
	Antinuclear antibody	12 (92%)	14 (88%)
	Anti-ENA	9 (70%)	7 (44%)
	Anti-DNA	1 (8%)	6 (38%)
	Anti-RNP	3 (23%)	3 (19%)
	Anti-SSA	4 (31%)	7 (44%)
	Anti-SSB	1 (8%)	2 (13%)
	Anti-Smith	2 (15%)	2 (13%)
	Anticardiolipin autoantibodies	3 (23%)	1 (16%)
	Lupus anticoagulant	6 (46%)	2 (13%)
	Anti-B2 glycoprotein	2 (15%)	0
ACR criteria	Malar rash	5 (38%)	8 (50%)
	Discoid lupus	2 (15%)	0
	Photosensitivity	5 (38%)	7 (44%)
	Ulcers	7 (54%)	7 (44%)
	Arthritis	12 (92%)	9 (56%)
	Serositis	4 (31%)	6 (38%)
	Lupus nephritis	4 (31%)	5 (31%)
	Neurological disorder	4 (31%)	0
	Hematologic disorder	6 (46%)	8 (50%)
	Immunologic disorder	9 (70%)	13 (81%)
	Antinuclear antibodies	13 (100%)	16 (100%)
Medication	Prednisone	9 (70%)	9 (56%)
	Azathioprine	3 (23%)	6 (38%)
	Methotrexate	1 (8%)	1 (16%)
	Hydroxychloroquine	11 (85%)	12 (75%)
	Mycophenolate mofetil	3 (23%)	2 (13%)

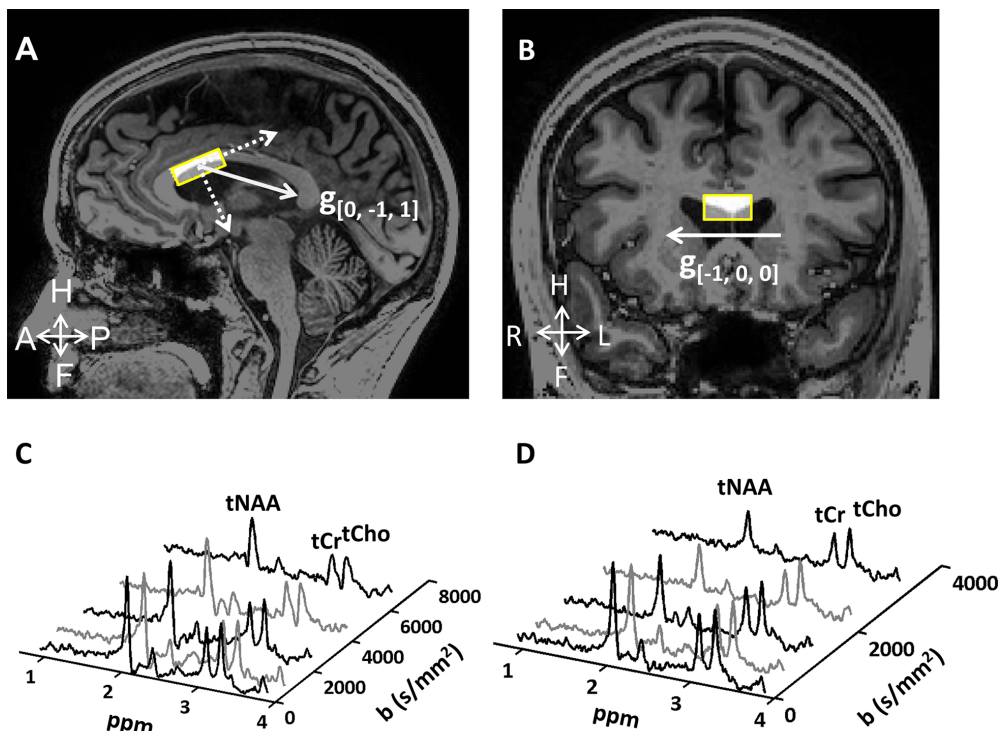


FIGURE 6.1: The position of the volume of interest in sagittal (a) and coronal (b) views. Gradients applied in directions approximately perpendicular (a) and parallel (b) to the callosal fibers are shown in solid lines. Typical spectra acquired with diffusion weighting in the $[0, -1, 1]$ and the $[-1, 0, 0]$ directions are shown as a function of b -value in panels (c) and (d), respectively. Line broadening of 5 Hz was applied for display purposes.

perpendicular to the callosal fibers (direction $[0, -1, 1]$), as shown in panels a and b of Figure 6.1.

The center frequency was set to the tNAA singlet peak at 2.0 ppm. Water suppression was performed using two frequency-selective excitation pulses, each followed by a dephasing gradient before metabolite excitation. Pencil beam second-order shimming was performed, resulting in a typical tNAA line width of 10 Hz. A peripheral pulse unit was used in order to gate data acquisition to the cardiac cycle, thereby minimizing signal fluctuations due to cardiac pulsation. The parameters for DW-MRS acquisitions were: TE = 121 ms, TR = 3 cardiac cycles (about 3000 ms), cardiac trigger delay = 300 ms, number of time-domain points = 1024, spectral width = 3000 Hz, gradient duration (δ) = 37 ms, bipolar gap = 16 ms, diffusion time (Δ) = 60.5 ms with 5 different gradient amplitudes resulting in b -values of 212, 651, 1335, 2262, and 3462 s/mm² in the $[-1, 0, 0]$ direction and 440, 1336, 2718, 4586, and 6945 s/mm² in the $[0, -1, 1]$ direction. The total number of spectra per diffusion condition was 32, resulting in a total scan time of 10~15 minutes. Following this scan, a shorter scan (fewer signal averages) with identical VOI

position and diffusion conditions was performed without water suppression and with the center frequency set at the water resonance frequency. These spectra were used for eddy-current correction in the post-processing stage.

6.2.3 DW-MRS Processing

All spectral pre-processing was performed with custom codes in MATLAB[®] release R2014b (Mathworks, Natick, MA, USA). Spectral pre-processing consisted of correcting DW-MRS data for eddy currents, zero-order phasing, correction of frequency drift for individual acquisitions, and removal of the residual water peak: averaged spectra were generated for each condition [27]. The resulting spectra were quantified with LCModel [28]. Cramér-Rao lower bound (CRLB) values were used to evaluate the quality of the spectra for each diffusion condition, and the acceptance threshold for DW-MRS data inclusion was set at $CRLB < 20\%$. Based on this, data sets from one NPSLE subject and one SLE subject were excluded from the tNAA analysis.

The LCModel spectral estimates were used to calculate the diffusivity (D_{par}) along the $[1, 0, 0]$ direction (roughly parallel to the callosal fibers) and diffusivity (D_{perp}) along the $[0, -1, 1]$ direction (roughly perpendicular to the callosal fibers) for tNAA, tCr and tCho. These were calculated by performing a linear fit of the natural logarithm of the DW-MRS signal amplitudes as a function of the diffusion weighting value b , assuming a monoexponential decay of the signal as a function of b in each direction:

$$\ln\left(\frac{S_{b,i}}{S_{b_0,i}}\right) = -b_i \cdot D_i \quad (6.1)$$

where $S_{b,i}$ is the measured signal in direction i , $S_{b_0,i}$ is the signal without diffusion weighting, b_i is the value of b in the direction i , and D_i is the calculated diffusion coefficient for direction i . Even though it is possible that the metabolite diffusion-weighted signal decay displays non-monoexponential behavior at very high values of b , our previous work has shown that in the range of b values used in this study the assumption of monoexponentiality is valid and diffusivity values are reproducible [12, 27]. An average of D_{par} and D_{perp} was calculated to assess the average diffusivity (D_{avg}) for tNAA, tCr and tCho. The quality of the linear fittings was evaluated via calculation of the coefficient of determination and an acceptance threshold was set at 75%, leading to exclusion of D_{avg} (tCr) values obtained from two NPSLE patients and one SLE patient.

6.2.4 Image Processing

DTI volumes were motion-corrected with ExploreDTI [29] and further processed with the DTI toolbox [30] of the FMRIB Software Library¹ to obtain the following DTI measures for each subject: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). These DTI metrics were further analyzed with tract based spatial statistics (TBSS) [31]. Statistical differences between NPSLE patients, SLE patients and HC were assessed in FA-MNI152 standard space using 5000 permutations and were corrected for multiple comparison based on threshold-free cluster enhancement [32]. One SLE patient data set was excluded due to poor registration to FA-MNI152.

T_1 -weighted images were used for tissue segmentation within the VOI [27]. FA maps were registered to the T_1 -weighted image of the same subject first by affine transformation

¹FSL release 5.0, <http://www.fmrib.ox.ac.uk/fsl/>

using FSL FLIRT [33, 34] and subsequently by non-rigid transformation using FNIRT [35]. The inverse transformation matrices generated were used to register the DW-MRS VOI to the DTI space. Subsequently, the registration procedure in TBSS was applied to transform each VOI to MNI152 space.

T_1 -weighted volumes were further processed in FreeSurfer² and the intracranial volume, total brain volume, center corpus callosum volume and mid-anterior corpus callosum volume were calculated for each subject. To evaluate whole brain and callosal atrophy due to SLE and NPSLE, total brain volume, center corpus callosum volume and mid-anterior corpus callosum volume were normalized according to the intracranial volume of the same subject.

6.2.5 Statistical analyses

All statistical analyses were performed with GraphPad Prism 5 for windows (version 5.01, GraphPad Software, USA). Unpaired t-tests were used to assess differences between two groups: a p-value < 0.05 was considered to represent statistically significant differences. Correlation between the metabolite D_{avg} measurements and clinical measures such as SLEDAI-2K score, SDI score and SLE duration were assessed with Pearson's correlation test.

6.3 Results

6.3.1 DW-MRS results

Figure 6.1 shows typical sets of diffusion-weighted spectra obtained with diffusion-weighting in the $[1,0,0]$ direction (c) and the $[0,-1,1]$ direction (d) from a healthy control subject. It can be seen that the attenuation of the tNAA peak is significantly more pronounced in the direction approximately parallel to the axonal fibers, along which diffusion of intra-axonal molecules is much less restricted than in the direction perpendicular to the fibers. Figure 6.2 shows the logarithm of the tNAA, tCho and tCr peak estimates from a DW-MRS data set of a healthy control subject as a function of b , and the linear fit to these data based on the assumption of monoexponential decay in both diffusion weighting directions. The strong anisotropy of the tNAA diffusion is reflected by the sharp difference between the decay rates in the two diffusion-weighting directions, in sharp contrast to both tCho, and tCr, for which the difference between the signal decay in the two directions is significantly smaller.

Figure 6.3 shows D_{avg} data of all three metabolites for HC, SLE and NPSLE patients. In NPSLE patients, $D_{avg}(tCho)$ and $D_{avg}(tCr)$ were significantly higher than in HC (p-value < 0.05 and p-value = 0.006, respectively). The difference in $D_{avg}(tNAA)$ values between NPSLE and HC was slightly above the threshold for statistical significance (p-value = 0.052). Average metabolite D_{avg} values for NPSLE, SLE and HC groups are shown in Table 6.2. No significant differences were observed in any metabolite D_{avg} between NPSLE and SLE or between SLE and HC. When all SLE patients, with and without past CNS involvement, were grouped together and compared to HC, $D_{avg}(tCr)$ remained significantly higher in SLE than in HC ($0.19 \pm 0.03 \mu m^2/s$ in SLE, $0.17 \pm 0.02 \mu m^2/s$ in HC, p-value < 0.05).

Average diffusion coefficients of each of the metabolites showed a link to disease activity in both the SLE and NPSLE patients. When all SLE patients were pooled together,

²<http://surfer.nmr.mgh.harvard.edu/>

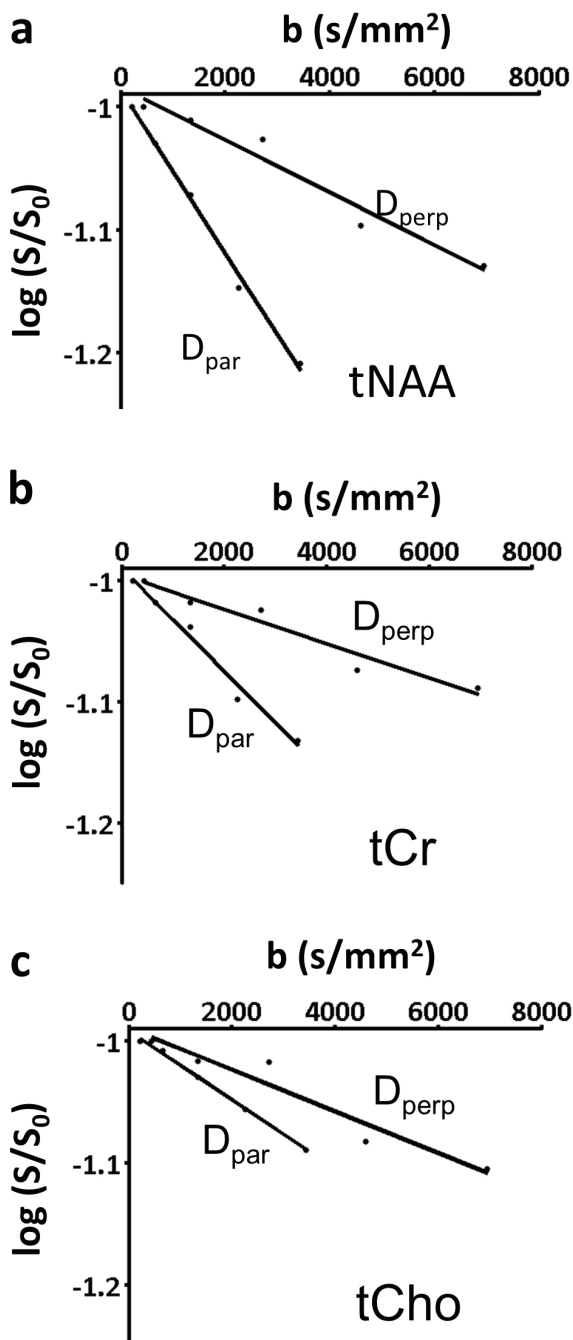


FIGURE 6.2: Logarithm of (a) tNAA, (b) tCr, and (c) tCho values from a single subject as a function of b-value. Linear fits used to extract parallel (D_{par}) and perpendicular (D_{perp}) metabolite diffusivities are shown by the solid lines.

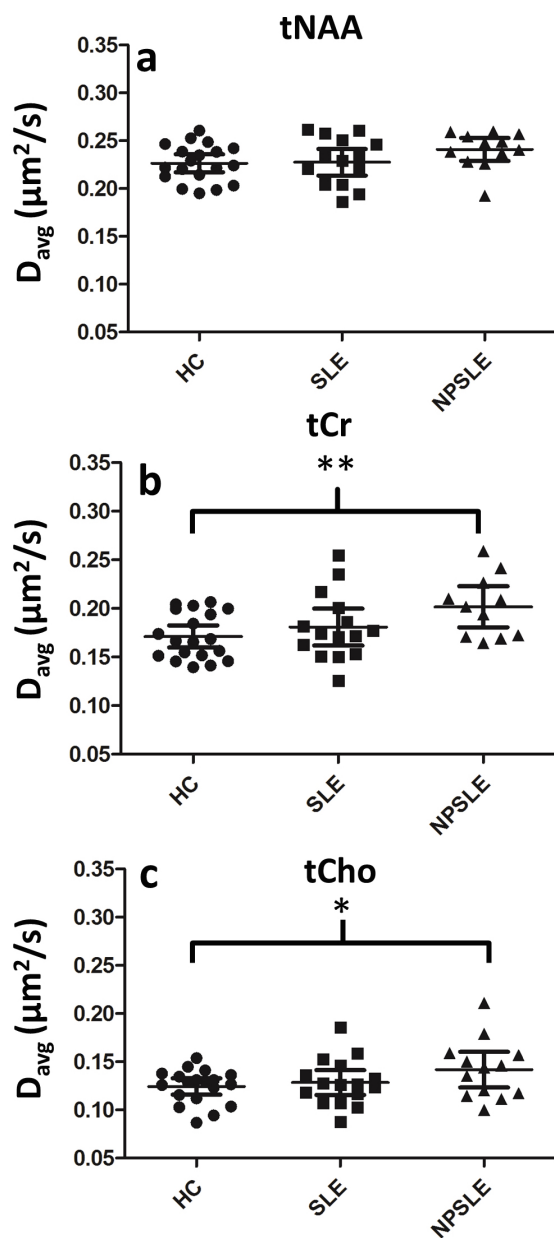


FIGURE 6.3: Metabolite D_{avg} values for healthy controls (HC), SLE patients and NPSLE patients. $D_{avg}(tNAA)$, $D_{avg}(tCr)$ and $D_{avg}(tCho)$ data are shown in panels (a), (b), and (c), respectively. Statistically significant differences with p -value < 0.05 are indicated by *, and by ** for p -value < 0.01 . No significant differences were found between SLE and HC, or between NPSLE and SLE in any of the metabolite D_{avg} values.

TABLE 6.2: Metabolite D_{avg} values for NPSLE patients, SLE patients and healthy controls.

	NPSLE patients (n=13)	SLE patients (n=16)	Healthy controls (n=19)	% increase in NPSLE vs HC
D_{avg} (tNAA) $\mu\text{m}^2/\text{s}$	0.24 ± 0.02	0.23 ± 0.02	0.23 ± 0.02	7%
D_{avg} (tCr) $\mu\text{m}^2/\text{s}$	$0.20 \pm 0.03^{**}$	0.18 ± 0.03	0.17 ± 0.02	18%
D_{avg} (tCho) $\mu\text{m}^2/\text{s}$	$0.14 \pm 0.03^*$	0.13 ± 0.02	0.12 ± 0.02	14%

regardless of their NP status, SLEDAI-2K scores positively correlated with D_{avg} (tNAA) (p-value = 0.045, $r = 0.39$), D_{avg} (tCr) (p-value = 0.021, $r = 0.45$) and D_{avg} (tCho) (p-value = 0.042, $r = 0.38$). Scatter plots of the SLEDAI-2K scores of all patients as a function of metabolite D_{avg} values are given in Figure 6.4.

When SLE and NPSLE patients categorized as SLE-active (SLEDAI-2K ≥ 8) were compared to HC, the differences in D_{avg} (tCr) and D_{avg} (tCho) were significant (D_{avg} (tCr) = $0.22 \pm 0.03 \mu\text{m}^2/\text{s}$, D_{avg} (tCho) = $0.15 \pm 0.03 \mu\text{m}^2/\text{s}$, p-value = 0.003 and p-value = 0.014, respectively), while the difference in D_{avg} (tNAA) between the two groups was only close to statistical significance (D_{avg} (tNAA) = $0.25 \pm 0.01 \mu\text{m}^2/\text{s}$, p-value = 0.051). No correlation was found between metabolite D_{avg} values and SLE duration or SDI scores.

6.3.2 DTI and volumetric results

Significantly lower FA, higher MD and higher RD in the NPSLE patient group compared to the HC group were found throughout white matter, including the DW-MRS VOI. Similar differences in FA, MD and RD were found between NPSLE and SLE patients. Figure 6.5 shows maps of statistically significant differences in DTI measures overlaid on the MNI152 T_1 -weighted image, and the cumulative DW-MRS VOI (i.e. the sum of all the VOIs which are positioned slightly differently for each patient). No significant differences were found in any DTI measure between SLE patients (with and without past CNS involvement) and HC. No significant differences were found in corpus callosum volumes or total brain volumes between SLE or NPSLE patients and HC.

6.4 Discussion

This is the first study to address cell-specific microstructural alterations in the brain of SLE patients with DW-MRS at ultrahigh field. This study focused on measuring the diffusion properties of two predominantly glial metabolites, tCr and tCho, and one exclusively axonal/neuronal metabolite, tNAA. The most salient finding in this study is the strong and consistent link between both D_{avg} (tCr) and D_{avg} (tCho) and disease state, with respect to disease activity and to past CNS involvement, suggesting glial involvement in the brain of these patients. Two potential pathological mechanisms that can explain the significantly higher tCr and tCho diffusivities found in NPSLE patients are inflammation-mediated morphological changes in microglia and astrocytes, and intracellular edema, which would affect both glia and neurons/axons.

Astrocytic and microglial reactivity in response to inflammation and/or ischemia are both highly consistent with an increase in intracellular diffusivity in glia. Reactivity-related cellular hypertrophy and thickening of the processes near the soma (especially in astrocytes) [36] would result in an increase of the intracellular space, and a decrease

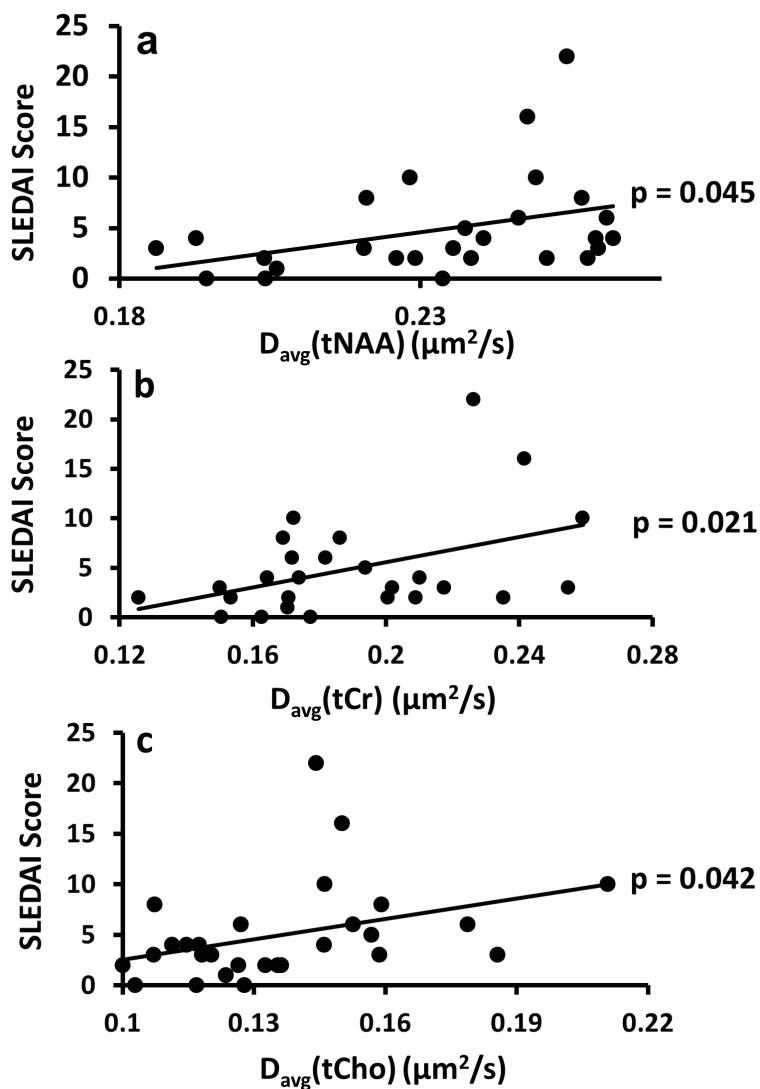


FIGURE 6.4: Correlation of metabolite D_{avg} values with patient SLE disease activity index 2000 (SLEDAI-2K) scores. The resulting linear regression line and significance of the correlations are shown for $D_{avg}(tNAA)$ (a), $D_{avg}(tCr)$ (b) and $D_{avg}(tCho)$ (c).

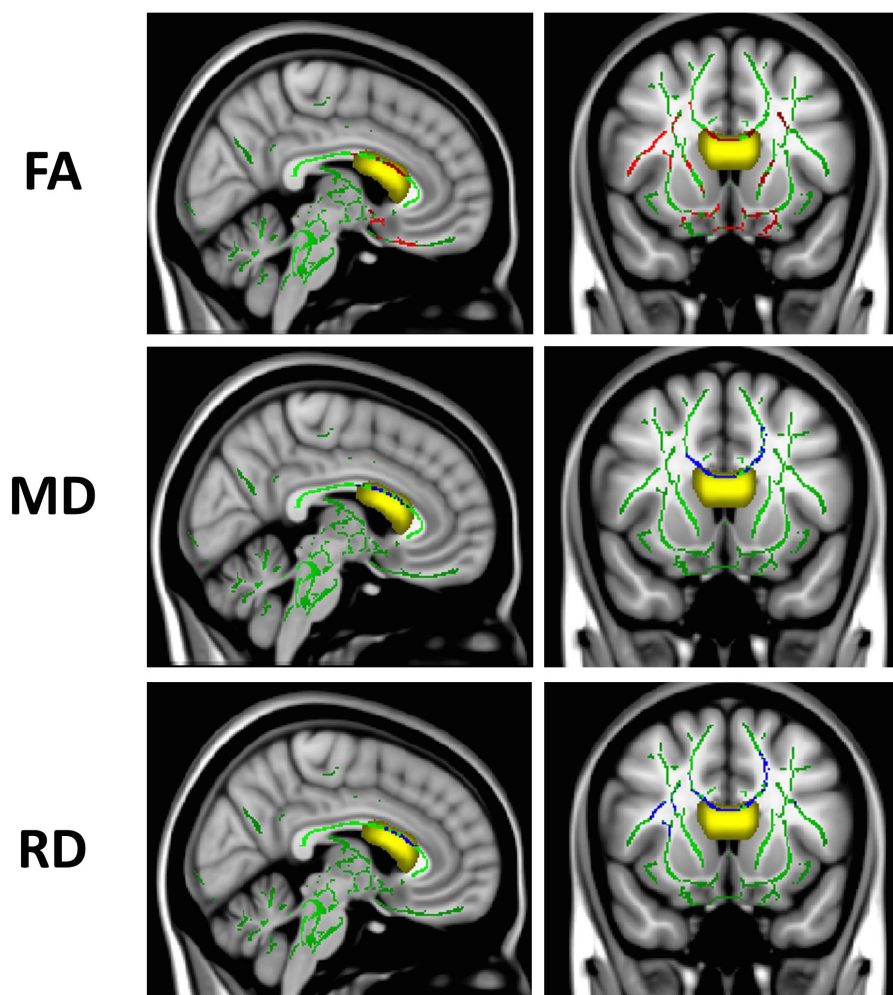


FIGURE 6.5: TBSS results showing regions with statistically significant differences in DTI measures in the white matter skeleton of NPSLE patients and healthy controls (p -value < 0.05). Maps are shown for one sagittal (left column) and one coronal (right column) slice in MNI152 space. The mean FA skeleton is shown in green, regions with higher values in the NPSLE patients compared to HC are shown in blue and regions with lower values in NPSLE patients compared to HC are shown in red. Cumulative VOIs chosen for DW-MRS of NPSLE patients and healthy controls are shown in yellow.

in molecular crowding and intracellular tortuosity, leading to increased diffusivity in the cytosol. The pathogenesis of NPSLE is thought to involve various immune and inflammatory processes that can lead to neuronal injury and vasculopathy [37]. The inflammatory response to injury results likely in glial reactivity and cellular hypertrophy, especially in microglia and astrocytes [38, 39]. Histopathological investigations of brains of NPSLE patients, confirm the widespread presence of reactive microglia and astrocytes, as well as of lipid-laden macrophages among the heterogeneous pathological phenomena [40]. Furthermore, the correlation of *in vivo* MRS results with histological results from the same patients [40] suggest: (a) an association between an increase in tCho concentrations and gliosis, vasculopathy and edema; (b) possible association of tCr with gliosis and reduced neuronal/axonal density; and (c) an association between lower tNAA concentrations and a decrease in neuronal/axonal density [40].

The higher $D_{\text{avg}}(\text{tNAA})$ values found in NPSLE patients compared to HC may be attributed to changes in cytosolic viscosity in axons due either to neuronal/axonal damage or to cytotoxic edema, both of which are seen in the histopathology of brains of NPSLE patients [8]. Higher $D_{\text{avg}}(\text{tNAA})$ has also been observed in a study of patients with schizophrenia where it was hypothesized that inflammatory processes may play a role [41]. On the other hand, *lower* tNAA parallel diffusivity values were found in multiple sclerosis (MS) patients compared to HC in a study focused on myelin and axonal changes in the corpus callosum [19]. It is likely that the different behaviors in tNAA diffusivity seen in MS and in NPSLE reflect different intra-axonal pathological mechanisms associated with these two diseases. Central to MS are demyelination and axonopathy [42, 43]. Since demyelination has no direct effect on diffusion in the intra-axonal space, it has been hypothesized that in MS the decrease in tNAA axosolic diffusivity stemmed from axonal damage that included unusual patterns of neurofilament phosphorylation and packing compared to normal tissue, and a less organized axoskeleton and/or problems with axonal transport [44]. In contrast to findings in MS, histology of NPSLE patients have shown that cerebral edema occurs much more frequently than axonal/neuronal loss [8] and is thus more compatible with the increase in axosolic diffusivity, as evidenced by the increase in $D_{\text{avg}}(\text{tNAA})$ observed in our study.

The high correlation in all SLE/NPSLE patients between SLE disease activity, as quantified by the SLEDAI-2K score, and $D_{\text{avg}}(\text{tCr})$ and $D_{\text{avg}}(\text{tCho})$ suggests that the SLE-related peripheral inflammation and autoimmune response may have effect on the brain, independent of overt clinical CNS involvement in SLE. Additionally, significant correlation of $D_{\text{avg}}(\text{tNAA})$ values with SLEDAI-2K scores suggests a permanent or continuous damage to axons correlated with high SLE activity. This is further corroborated by the finding that patients with higher disease activity (those we defined as SLE-active) have higher metabolite diffusivity levels and higher statistical significance in the difference in metabolite diffusivity compared to HC. A previous MRS study in NPSLE/SLE has shown a significantly lower tNAA/tCr level in SLE patients with a high SLEDAI-2K score, and that the level of tNAA/tCr was renormalized in follow-up for patients who were no longer SLE-active, regardless of their NP status [11]. This finding suggests a pathological mechanism, attributed by the authors to neuronal dysfunction, that affects both neurons and axons, the degree of which depends on SLE disease activity, but which is essentially reversible in nature. In our view, and based on our corroborative findings, we attribute this finding to intracellular/intraaxonal edema. It has been suggested that inflammation outside the brain can prime microglia and result in microglial activation for several weeks

[45]. Our findings, as well as those described by Appenzeller et al. [46] support the view that systemic inflammation affects the brain in NPSLE, and the underlying mechanism by which this occurs, e.g. potential disruption of the blood brain barrier in SLE [47], should be further investigated.

Metabolite ADC values found in HC in this study are similar to those reported in previous DW-MRS studies performed on a similar region of the corpus callosum at 7 Tesla and 3 Tesla [13, 27]. A recently published robustness and reproducibility study of DW-MRS in the anterior body of the corpus callosum, aimed to provide guidelines for DW-MRS acquisition for clinical studies such as the one presented here. Using power calculations based on actual data it was estimated that in order to observe a 10% difference in D_{avg} values for tNAA, tCr and tCho in a case-control study, nine, four and twelve subjects, respectively, are sufficient [27]. In this current study we observed a 7% increase in D_{avg} (tNAA), 19% increase in D_{avg} (tCr) and 14% in D_{avg} (tCho) in NPSLE patients compared to HC, with thirteen and nineteen subjects per group respectively. This suggests that it is feasible to observe reliably the disease effect on metabolite D_{avg} reported here. Moreover, based on our power estimation, the number of subjects required to observe reliably a difference in D_{avg} (tCho) is higher than that required for D_{avg} (tNAA). This further supports the notion that our findings indicate a larger effect size for glial than for axonal involvement in SLE/NPSLE. The DTI results are also consistent with previous studies on SLE and NPSLE [5, 6, 48]. Atrophy measurements did not show any significant corpus callosum or total atrophy in the patient population, which could be a type II error due to the small size of the cohort.

There were several challenges and limitations to the study. The main (unmet) challenge of this study was to scan patients with active NP symptoms at the time of the scan, as well as to include more patients with high SLE disease activity. Low incidence of active NPSLE was a factor, as well as significant potential discomfort to patients with clinically overt NP symptoms, preventing these patients from being involved in a research study that is not part of the routine clinical procedure. In our patient cohort, the relative low number of patients with antiphospholipid syndrome made it difficult to evaluate the effect of ischemic/vascular changes on metabolite diffusion. Future studies will focus on separate evaluation of the effects of NP activity, ischemic and inflammatory effects. Missing in this study is an evaluation of the concentrations of the different metabolites, and the study would have benefitted from a separate MRS acquisition at short TE, for an accurate evaluation of the metabolite concentrations in the same VOI, together with additional cell-specific metabolites such as glutamate, glutamine and myo-inositol.

6.5 Conclusions

The results presented in this paper show for the first time that intracellular metabolite diffusion reflective of glial and neuronal/axonal involvement can be measured in a complex autoimmune inflammatory disorder such as SLE/NPSLE, and can thus help unravel pathogenic mechanisms in this complex disease. To our knowledge, DW-MRS of brain metabolites is the only clinical neuroimaging method capable of non-invasive cell-specific detection of cellular morphological changes in disease. We believe that the DW-MRS technique has great potential for the study of the etiology of disease-related changes in tissue microstructure, and that its importance in clinical applications will continue to grow if incorporated in a comprehensive diagnostic scanning protocol together with existing microstructural MRI tools, such as DTI, MTI and susceptibility weighted

imaging.

6.6 References

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