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Original Research Paper

Fatigue in multiple sclerosis: Associations with clinical, MRI and CSF parameters

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Abstract

Background: Damage of different brain structures has been related to fatigue. Alternatively, functional alterations of central nervous system (CNS) cells by the inflammatory milieu within the CNS may be responsible for the development of fatigue.

Aim: To investigate the effect of structural brain damage and inflammatory cerebrospinal fluid (CSF) changes on fatigue in multiple sclerosis (MS).

Methods: We determined the association of different clinical, CSF and magnetic resonance imaging (MRI) parameters with prevalence and severity of fatigue, as measured by the Fatigue Scale for Motor and Cognitive Functions in 68 early MS patients (discovery cohort). We validated our findings in two MS cohorts: the MRI validation cohort ($N=233$) for the clinical and MRI parameters, and the CSF validation cohort ($N=81$) for the clinical and CSF parameters.

Results: Fatigue was associated with clinical disability. Fatigue did not correlate with any CSF parameter but correlated negatively with total and cortical grey matter volume. However, when controlling for Expanded Disability Status Scale (EDSS) in a multivariate model, these associations lost significance.

Conclusion: Disability and disease duration best explain fatigue severity but none of the tested MRI or CSF parameter was reliably associated with fatigue.

Keywords: Multiple sclerosis, demyelinating diseases, fatigue, magnetic resonance imaging, flow cytometry, cerebrospinal fluid

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Introduction

Fatigue is one of the most prevalent symptoms in multiple sclerosis (MS) patients. It is present early in the disease course,[1,2] can even precede other MS symptoms[3] and predicts disease progression.[1,4]

The pathophysiology of fatigue is still unclear. Damage of specific brain structures and strategic localization of MS lesions have been related to fatigue in MS patients. However, results from previous studies are conflicting.[5] Alternatively, the inflammatory milieu in the central nervous system (CNS) compartment of MS patients might produce symptoms of fatigue by inducing functional alterations of CNS intrinsic cells. This hypothesis is supported by the fact that other autoimmune diseases, chronic or acute infections and malignancies are characterized by fatigue similar to MS.[5] Studies investigating immunologic markers for fatigue in the blood of MS patients failed to identify a meaningful parameter.[5] Inflammatory mechanisms may mainly occur in the CNS compartment and might not be measurable in blood samples of MS patients.[5]

In the study at hand, we tested possible associations of fatigue with the morphometric imaging parameters suggested until now[5] and immunologic markers in a cohort of 68 early MS patients. In a second step, we controlled for clinical disability and depression which are both associated with fatigue.[6]

Immunologic markers were investigated in the cerebrospinal fluid (CSF) compartment, which might reflect inflammatory changes in the CNS parenchyma

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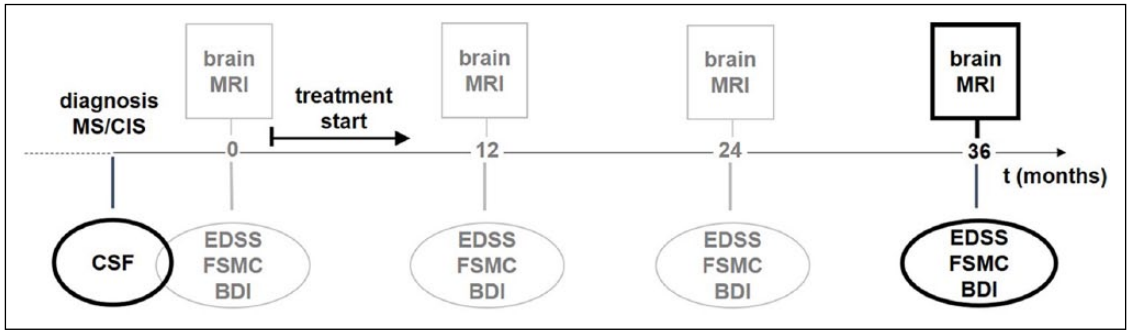

Figure 1. Study design.
A schematic overview of the study design is given. CSF is withdrawn at disease onset before the start of an immunomodulatory treatment. Afterwards, standardized brain MRI scans, clinical and neuropsychological tests are performed annually. Focusing on 3-year follow-up, three cohorts were investigated: discovery cohort, patients with CSF analysis at disease onset, clinical and MRI follow-up data; CSF validation cohort, patients with CSF analysis at disease onset and clinical follow-up data; and MRI validation cohort, patients with brain MRI and simultaneous clinical tests.
BDI: Becks Depression Inventory II; CIS: clinically isolated syndrome; CSF: cerebrospinal fluid; EDSS: Expanded Disability Status Scale; FSMC: Fatigue Scale for Motor and Cognitive Functions; MRI: magnetic resonance imaging; *t*: time.

to a certain degree. We were particularly interested in the distribution of CSF immune cells, as immunologic dysregulation[7] with alterations in B-[8] and T-[9,10] cell function had also been described in patients with chronic fatigue syndrome (CFS). B-cell depleting therapies have been shown to ameliorate fatigue in CFS[11] and autoimmune diseases[12] but did not influence fatigue in a trial with 75 MS patients.[13] We aimed to validate our findings in two separate cohorts of early MS patients, the magnetic resonance imaging (MRI) and CSF validation cohort.

Materials and methods

Subjects and study design

This study was performed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and was approved by the local ethics committee. We retrospectively analysed data that were collected in an observational study (TUM-MS) since January 2009 at the Department of Neurology at the Technical University of Munich. The study design is as follows (see also Figure 1): All patients with a diagnosis of MS according to current McDonald criteria[14] or clinically isolated syndrome (CIS) that are regularly followed in our MS outpatient clinic were asked for their informed consent to participate in the TUM-MS study and to provide their data and biomaterials for scientific purposes. CIS was defined as first demyelinating event suspicious of MS accompanied by at least two brain white matter (WM) lesions detected by MRI. If disease diagnosis was made in our clinic, CSF was drawn as part of routine clinical work-up. After study inclusion, standardized brain MRI scans, clinical and neuropsychological tests were performed annually within 30 days. Disability was quantified by Expanded Disability Status Scale (EDSS) by the treating neurologist in the MS outpatient unit; fatigue was assessed by Fatigue Scale for Motor and Cognitive Functions (FSMC). FSMC is a 20-item questionnaire developed to differentiate grade motor and cognitive fatigue in MS patients.[15] Depression was evaluated by Beck Depression Inventory-II (BDI).[16]

For the study at hand, we excluded patients with relevant comorbidities as malignancies, autoimmune and psychiatric diseases. Patients with depression were included, as depression affects around 25% of MS patients with a lifetime prevalence of up to 54%.[17] Excluding these patients might introduce a systematic bias. Nevertheless, one patient with long-standing major depression before the onset of MS symptoms was excluded. We also excluded patients with a relapse or EDSS >4 at time of fatigue assessment. For CSF analysis, only therapy-naïve patients were selected (no immunomodulatory drugs, no corticosteroids within the past 30 days). Afterwards, they received different immunomodulatory drugs according to the recommendation of their treating physician. We focused on fatigue scores 3 years after disease diagnosis and investigated three cohorts of MS patients: The discovery cohort consisted of 68 patients of whom all investigated items were available (CSF at disease onset, follow-up brain MRI and clinical tests). The MRI validation cohort consisted of 233 patients of whom brain MRI scans with simultaneous clinical and neuropsychological tests were available. The CSF validation cohort consisted of 81 patients of whom CSF at disease onset and follow-up clinical tests were available.

Table 1. Basic data and clinical examination.

Basic data	Discovery cohort	MRI validation cohort	CSF validation cohort
Number	68	233	81
Gender (male; female)	19; 49	78; 155	30; 51
Age at first manifestation (years; mean ± SD)	32.5 ± 8.9	33.1 ± 10.0	33.0 ± 8.3
Clinical examination			
Age (years; mean ± SD)	36.8 ± 9.3	37.8 ± 10.1	37.1 ± 8.7
Disease duration (years; mean ± SD)	4.3 ± 3.6	5.0 ± 4.5	4.1 ± 3.6
Disease course (CIS; RRMS; SPMS)	7; 60; 1	34; 199; 0	9; 71; 1
Time span CSF – clinical test (months; mean ± SD)	33.6 ± 6.3	–	30.8 ± 11.3
Disease modifying drugs	None: 15; IFN: 19; GA: 14; DMF: 7; FTY: 9; Nat: 2; RTX: 1; TFN: 1	None: 66; IFN: 90; GA: 33; DMF: 16; FTY: 18; Nat: 6; RTX: 1; TFN: 3	None: 20; IFN: 35; GA: 15; DMF: 6; FTY: 2; Nat: 3
FSMC cognition (mean ± SD)	20.1 ± 10.4	21.5 ± 10.3	20.9 ± 11.3
FSMC motor (mean ± SD)	20.8 ± 10.6	22.6 ± 10.6	20.9 ± 10.8
FSMC total (mean ± SD)	40.8 ± 20.5	44.1 ± 20.5	41.7 ± 21.9
EDSS (median; range)	1.0; 0–4	1.5; 0–3.5	1.0; 0–4
BDI (mean ± SD)	6.2 ± 6.8	7.1 ± 7.4	5.7 ± 6.1

BDI: Becks Depression Inventory-II; CIS: clinically isolated syndrome; DMF: dimethyl fumarate; EDSS: Expanded Disability Status Scale; FSMC: Fatigue Scale for Motor and Cognitive Functions; FTY: fingolimod; GA: glatiramer acetate; IFN: beta interferon; Nat: natalizumab; RRMS: relapsing-remitting multiple sclerosis; RTX: rituximab; SD: standard deviation; SPMS: secondary progressive multiple sclerosis: TFN: teriflunomide.

Patient characteristics, clinical and neuropsychological tests at follow-up are summarized in Table 1.

Analysis of CSF

Routine CSF parameters

CSF was drawn as part of routine clinical work-up. Peripheral blood was taken immediately after lumbar puncture. CSF cells were counted manually in a Fuchs Rosenthal chamber. CSF glucose and lactate levels were measured by an enzymatic-amperometric method using chip-sensor technology (EKF Biosen C-Line). Albumin CSF/serum ratios and intrathecal immunoglobulin synthesis were determined by nephelometry (Siemens ProSpec®). Fluorescence-associated cell sorting (FACS) of CSF immune cells was performed as described previously.[18] The following antibodies were used for staining: cluster of differentiation (CD) 45, V450; CD3, APC-Cy7; CD4, PerCP (all BD Bioscience); CD8, PE-Cy7; CD14, FITC; CD19, ECD; CD138, PE; and CD56, APC (all Beckman Coulter). We stained CD4+ T cells (CD45 + CD3 + CD4 +), CD8 + T cells (CD45 + CD3 + CD8+), monocytes (CD45+CD14+), natural killer (NK) cells (CD45+CD56+), B cells (CD45+CD19+CD138−) and plasmablasts (CD45+CD19+CD138+). The percentage of each subpopulation was determined in relation to all CD45 positive single cells using FlowJo (version 10.1). In the discovery cohort, B cells and plasmablasts staining was missing in seven patients and monocytes staining was missing in two patients. CSF parameters are summarized in Table 2.

MRI

Scanning protocol

All brain images were acquired on the same 3T scanner (Achieva, Philips, The Netherlands). The scanning protocol included 3D GRE T1-weighted (w) sequence (orientation: 170 contiguous sagittal 1-mm slices; field of view: $240 \times 240\,mm^2$; voxel size: $1.0 \times 1.0 \times 1.0\,mm^3$; repetition time (TR): 9 ms; echo time (TE): 4 ms), and 3D FLAIR sequence (orientation: 144 contiguous axial 1.5-mm slices; field of view: $230 \times 185\,mm^2$; voxel size: $1.0 \times 1.0 \times 1.5\,mm^3$; TR: 10^4 ms; TE: 140 ms; TI: 2750 ms).

Table 2. CSF analysis.

CSF analysis	Discovery cohort	CSF validation cohort
Number	68	81
Relapse within 30 days before spinal tap (yes; no; unknown)	54; 14; 0	67; 11; 3
Cell count count/µL; mean ± SD Norm: <3/µL	10.4 ± 16.9	12.3 ± 17.7
Glucose (mg/dL; mean ± SD) Norm: 30–80 mg/dL	59.0 ± 8.3	61.4 ± 9.4
Lactate (mmol/L mean ± SD) Norm: <2.5 mmol/L	1.6 ± 0.3	1.6 ± 0.3
Albumin ratio 10^{-3} (mean ± SD) Norm: <6.5	6.5 ± 3.2	6.1 ± 2.4
Intrathecal IgG synthesis (no; yes; mean ± SD)	32; 36; 35.2 ± 21.4	42; 39; 35.7 ± 19.4
Intrathecal IgA synthesis (no; yes; mean ± SD)	61; 7; 25.3 ± 25.9	78; 3; 36.7 ± 29.8
Intrathecal IgM synthesis (no; yes; mean ± SD)	61; 7; 34.9 ± 16.9	67; 14; 33.1 ± 21.0
Oligoclonal bands (no; intermediate; yes)	7; 12; 49	6; 9; 66
CD4 + T cells (% of CD45+ cells; mean ± SD)	66.7 ± 8.2	65.4 ± 9.6
CD8+ T cells (% of CD45+ cells; mean ± SD)	18.3 ± 5.5	17.5 ± 6.2
CD4+/CD8 ratio (mean ± SD)	4.1 ± 1.7	4.3 ± 2.0
CD19+ B cells (% of CD45+ cells; mean ± SD)	3.0 ± 2.8 (N = 62)	2.8 ± 1.9
CD19+CD138+ plasmablasts (% of CD45+ cells; mean ± SD)	0.6 ± 0.6 (N = 62)	0.6 ± 0.6
CD56+ natural killer cells (% of CD45+ cells; mean ± SD)	2.7 ± 1.4	2.1 ± 1.1
CD14+ monocytes (% of CD45+ cells; mean ± SD)	2.5 ± 3.6 (N = 66)	2.0 ± 5.2

Norm: normal values; Ig: immunoglobulin; SD: standard deviation; CSF: cerebrospinal fluid.

T2-hyperintense WM lesions

T2-hyperintense WM lesions were segmented from FLAIR and T1-w images by a lesion growth algorithm as implemented in the lesion segmentation tool (LST) toolbox version 2.0.15 (www.statistical-modelling.de/lst.html) for SPM12 (http://www.fil.ion.ucl.ac.uk/spm). Lesion maps were normalized before extraction of lesion volumes. Brainstem and cerebellar WM lesion volumes were determined by masking with brainstem and cerebellar WM masks from LONI Probabilistic Brain Atlas (LPBA40).[19] Lesions were filled with normal appearing WM intensities in T1-w images by LST.[20] A natural logarithmic transformation was applied to all lesion volumes to approximate normal distribution.

Segmentation of brain compartments

Brain compartments were segmented with the computational anatomy toolbox (CAT12, version 916, http://dbm.neuro.uni-jena.de/cat/) as implemented in SPM12. We used SPM12 version 6685 for our analysis. We run the segmentation pipeline with the default settings that proceeds as follows: Lesion-filled T1-w images are normalized to Montreal Neurological Institute (MNI) template, segmented into the tissue

classes of grey matter (GM) and WM, and corrected for signal inhomogeneities (correction of bias-field). Segmented images are scaled with the amount of volume changes resulting from normalization (modulation).[21] Before extraction of brain compartments, all voxels, classified as lesions by LST, were set to 0 in the GM image and to 1 in the WM image in order to correct for possible lesion filling errors.

Regional volumes were extracted from GM and WM images with the following atlases: LPBA40[19] for putamen, caudate nucleus, cerebellar cortex, brainstem and cerebellar WM; neuromorphometrics atlas (http://neuromorphometrics.com/) for thalamus, hippocampus and accumbens nucleus; and CoBrA[22] for amygdala. For cerebral cortex, we used an own mask.[23] Total intracranial volume (TIV) was estimated by a reverse brain mask method.[24] All MRI parameters are summarized in Table 3.

Voxel-wise analysis

In case of significant associations of global brain volumes with fatigue, we performed voxel-wise correlation in SPM12. Therefore, normalized GM images were smoothed with an 8-mm Gaussian kernel. Lesion maps are normalized by applying the deformation matrix derived from the corresponding lesion-filled T1-w image and smoothed with a 12-mm Gaussian kernel. Analysis of GM images was corrected for age and, in a second step, for EDSS. We used a False Discovery Rate of <0.05 to account for multiple testing.

Quality assurance and plausibility control

Quality of standard CSF parameters was assured by regular interlaboratory tests. For plausibility control of FACS data, we confirmed the correlation of CSF plasmablasts count with intrathecal IgG synthesis[18] in the discovery ($r=0.432$; $p<0.0001$) and the CSF validation cohort ($r=0.515$; $p=0.001$). MRI data were checked by correlating age,[25] EDSS[26] and WM lesion volume[27] with different GM volumes (Figure 2).

Statistical analysis

We used IBM SPSS Statistics version 23 and R-environment for statistical computing[28] to analyse and plot data. We investigated the effect of demographic and clinical parameters on fatigue severity by Pearson's correlation analysis (age, disease duration, EDSS and BDI), unpaired *t*-test (gender) and analysis of variance (ANOVA; disease modifying drugs; categorical variable for each drug). We performed partial correlation analyses with FSMC and the extracted brain MRI parameters, correcting for age, gender and total intracranial volume. We performed partial correlation analyses with all CSF parameters, correcting for age and gender. To consider multicollinearity and to correct for multiple testing, a joint analysis of the effect of all CSF and MRI variables on FSMC was performed by elastic net regularized regression approach. This approach uses a mixture of L1 (Lasso regression) and L2 penalty (Ridge regression), thus it is able to perform shrinkage and variable selection simultaneously. For estimation, we used the EBglmnet package (https://CRAN.R-project.org/package=EBglmnet)[28] which implements an empirical Bayes approach. Optimal mixture parameters for the penalties were estimated using a leave-one-out cross validation.

Results

Prevalence of fatigue and association with clinical parameters

Motor and cognitive fatigue were highly correlated (all $R^2 \geq 0.82$; $p<0.0001$). In the following, we will report the results for the FSMC sum score only.

FSMC correlated significantly with disability (EDSS) in all three cohorts (discovery, MRI and CSF validation cohorts; $r=0.54$, 0.46 and 0.35, respectively; all $ps<0.0001$). All correlations remained significant when age and gender were included as confounders.

The distribution of BDI scores and the association with fatigue are illustrated in Figure 3. All patients with moderate or severe depression also had symptoms of fatigue, but not vice versa. Fatigue correlated significantly with BDI scores (discovery, MRI and CSF validation cohorts, $r=0.77$, 0.66 and 0.73, respectively; all $ps<0.0001$). We decided to repeat some of the following correlation analyses after exclusion of patients with moderate and severe depression to ensure that these outliers were not primarily responsible for the observed correlation.

In the MRI validation cohort, fatigue increased significantly with disease duration ($r=0.17$, $p=0.008$). The discovery cohort and the CSF validation cohort were not suitable for correlation of fatigue with disease duration as the clinical examination was performed at a standardized interval after diagnosis.

The immunomodulatory therapy did not have a significant effect on fatigue in any cohort (all $ps > 0.2$).

Table 3. Morphometric brain volume measures.

MRI data	Discovery cohort	MRI validation cohort
Number	68	233
Total intracranial volume (mL; mean ± SD)	1091.6 ± 99.7	1368.8 ± 128.0
Grey matter (mL; mean ± SD)	601.7 ± 50.2	609.0 ± 58.3
Cerebral cortex (mL; mean ± SD)	555.5 ± 47.0	562.4 ± 54.2
Putamen (mL; mean ± SD)	8.5 ± 1.0	8.6 ± 1.1
Caudate nucleus (mL; mean ± SD)	6.5 ± 0.8	6.5 ± 0.9
Thalamus (mL; mean ± SD)	9.8 ± 1.3	9.7 ± 1.6
Hippocampus (mL; mean ± SD)	6.2 ± 0.6	6.2 ± 0.6
Amygdala (mL; mean ± SD)	3.0 ± 0.3	3.0 ± 0.3
Accumbens nucleus (mL; mean ± SD)	0.8 ± 0.1	0.8 ± 0.1
Cerebellar cortex (mL; mean ± SD)	85.4 ± 7.5	86.0 ± 8.5
White matter (mL; mean ± SD)	497.6 ± 55.0	503.7 ± 52.8
Supratentorial white matter (mL; mean ± SD)	429.6 ± 49.4	435.8 ± 47.4
Brainstem white matter (mL; mean ± SD)	27.7 ± 2.9	27.7 ± 2.8
Cerebellar white matter (mL; mean ± SD)	40.3 ± 4.6	40.2 ± 4.7
White matter lesions (mL; mean ± SD)	6.5 ± 9.7	6.7 ± 10.6
Supratentorial white matter lesions (mL; mean ± SD)	6.5 ± 9.7	6.7 ± 10.6
Brainstem white matter lesions (mL; mean ± SD)	0.01 ± 0.03	0.01 ± 0.07
Cerebellar white matter lesions (mL; mean ± SD)	0.01 ± 0.04	0.01 ± 0.04

SD: standard deviation.

MRI measures and fatigue

Correlation analysis of fatigue with all extracted MRI parameters did not indicate any significant correlation in the discovery cohort. As significant correlations have been described in other studies,[5] we decided to repeat the analysis in the MRI validation cohort in order to increase statistical power. We found significant negative correlations of motor fatigue with volume of total ($r=-0.139$, $p=0.036$) and cortical ($r=-0.141$, $p=0.033$) GM. Exclusion of patients with moderate or severe depression did not change these results (GM: $r=-0.166$, $p=0.016$; cortical GM: $r=-0.164$, $p=0.017$). None of these correlations remained significant after additional correction for EDSS or disease duration (Figure 4). Likewise, in the multivariate model, fatigue was significantly associated with EDSS and BDI only, both in the discovery and the MRI validation cohort (all $ps<0.01$).

Voxel-wise analysis of the MRI validation cohort confirmed this finding: We identified GM clusters that were significantly associated with fatigue in right insular cortex, left and right occipital pole, left inferior parietal lobule, left inferior temporal gyrus, left precentral gyrus, right middle frontal gyrus and right precuneus

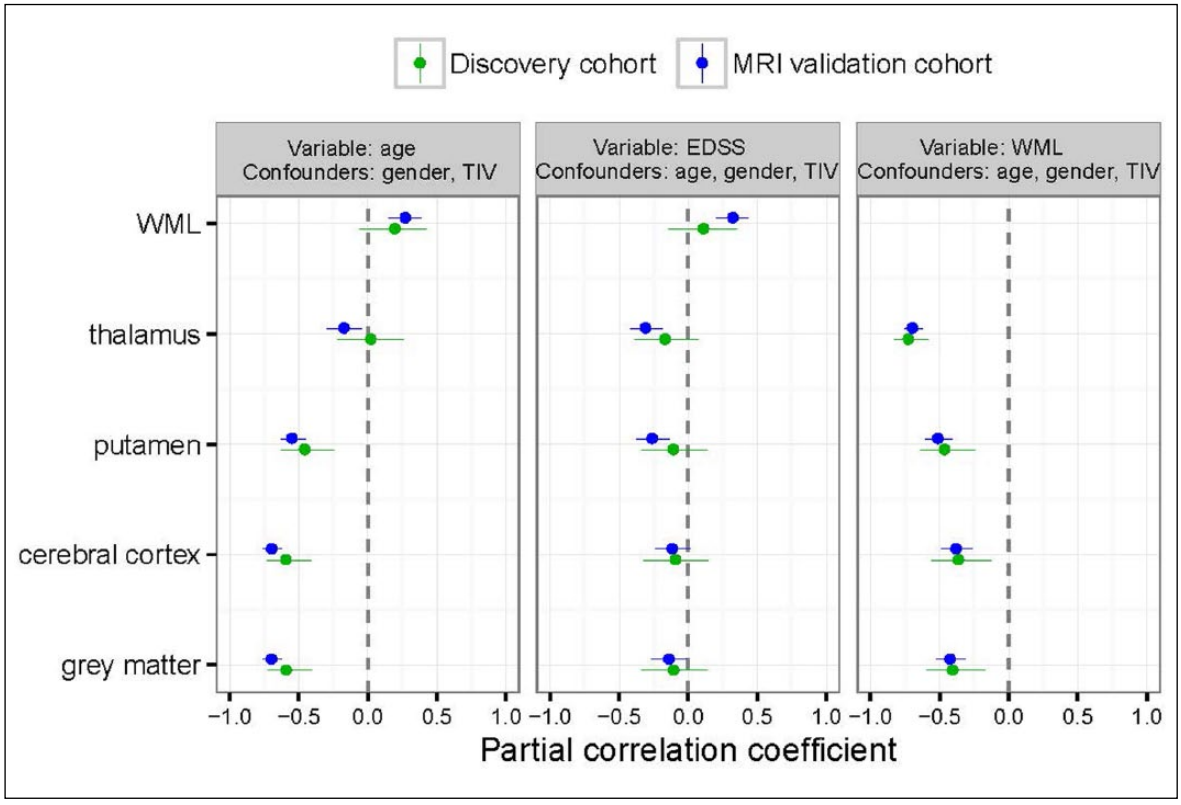

Figure 2. Plausibility control of MRI data by partial correlations.
Plausibility control of MRI data for the discovery cohort (green) and the MRI validation cohort (blue). Partial correlation coefficients and 95% confidence intervals are shown. Age was correlated with total white matter lesion volume and different grey matter structures, controlling for gender and total intracranial volume (left panel). EDSS (middle) and white matter lesion volume (right panel) were correlated with total white matter lesion volume and different grey matter structures, controlling for age, gender and total intracranial volume.
EDSS: Expanded Disability Status Scale; MRI: magnetic resonance imaging; TIV: total intracranial volume; WML: T2-hyperintense white matter lesion volume.

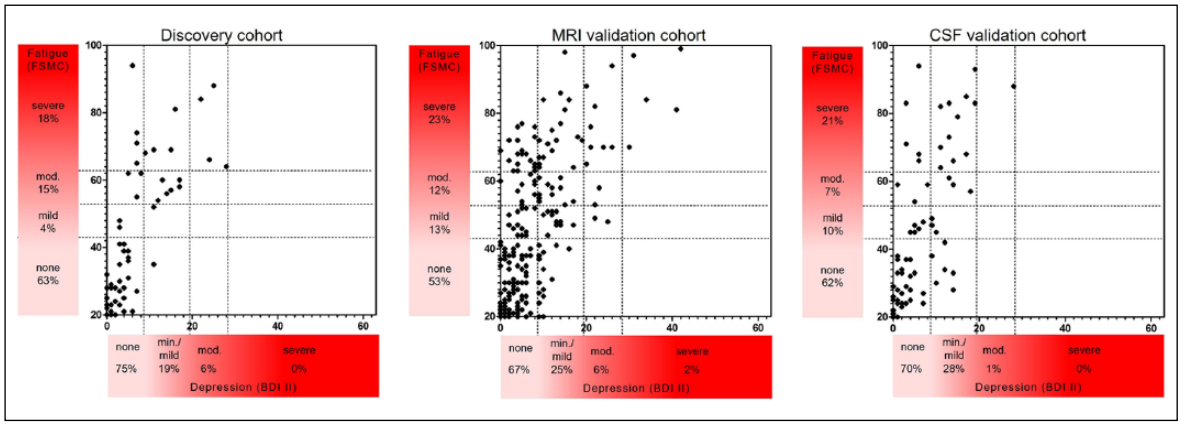

Figure 3. Prevalence of fatigue and depression.
The relationship of fatigue (Fatigue Scale for Motor and Cognitive Functions) with depression (Becks Depression Inventory-II) is shown for the discovery cohort (left panel), the MRI validation cohort (middle panel) and the CSF validation cohort (right panel).
BDI: Becks Depression Inventory-II; FSMC: Fatigue Scale for Motor and Cognitive Functions; min.: minimal; mod.: moderate.

cortex (all at a false discovery rate of <0.05), but none of them remained significant after inclusion of EDSS in the model. Voxel-wise analysis of lesion maps with fatigue did not show any significant cluster.

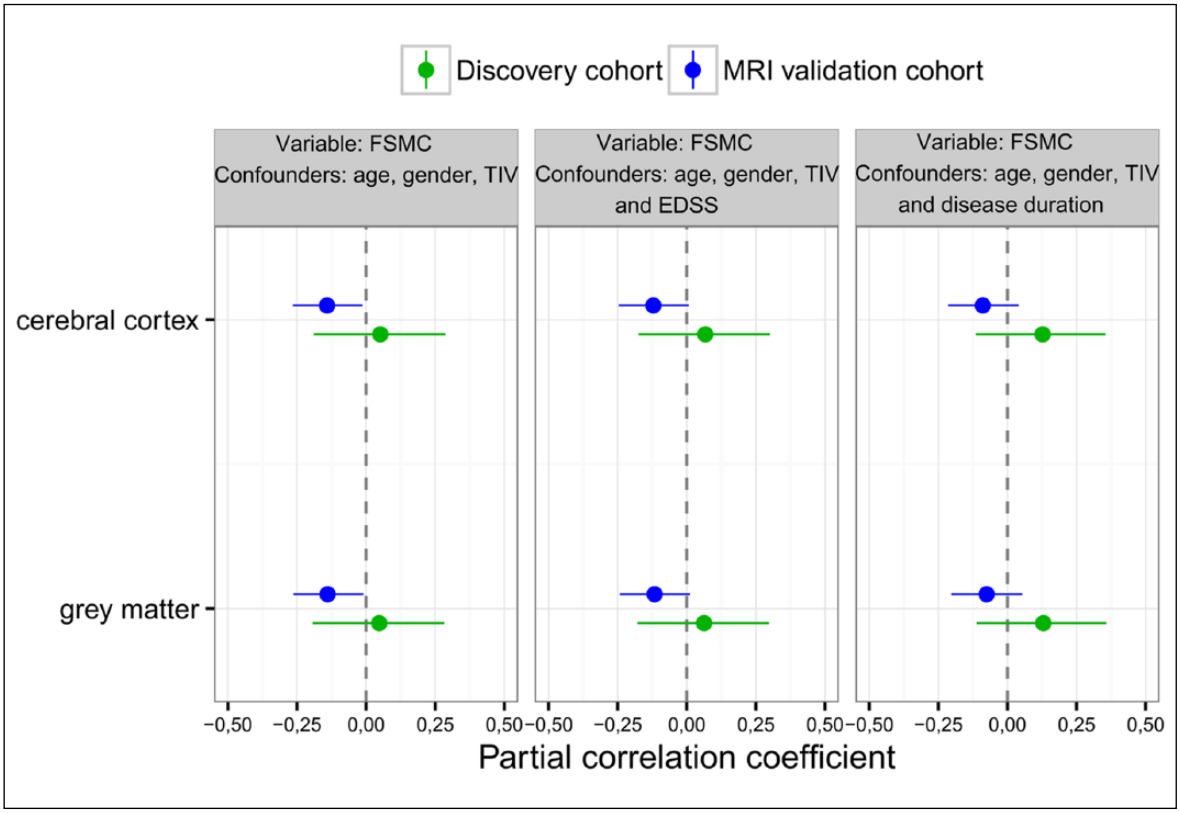

Figure 4. Partial correlation analyses of MRI parameters with fatigue.
Partial correlation coefficients and 95% confidence intervals of fatigue (Fatigue Scale for Motor and Cognitive Functions) with MRI parameters are shown for the discovery cohort (green) and the MRI validation cohort (blue). Only MRI parameters with a significant correlation in any analysis are shown. Confounders were age, gender and total intracranial volume (left panel); age, gender, total intracranial volume and Expanded Disability Status Scale (middle panel); and age, gender, total intracranial volume and disease duration (right panel).
EDSS: Expanded Disability Status Scale; FSMC: Fatigue Scale for Motor and Cognitive Functions; TIV: total intracranial volume.

CSF measures and fatigue

In the discovery cohort, none of the humoral CSF parameters was associated with fatigue. CSF CD4/CD8 ratio showed a trend towards negative correlation with fatigue ($r=-0.234$, $p=0.059$), which was not influenced by additional correction for relapse at time point of spinal tap (yes/no) and exclusion of patients with moderate and severe depression ($r=-0.235$, $p=0.068$). However, this finding could not be replicated in the CSF validation cohort. Here, CSF CD4/CD8 ratio showed a trend towards positive correlation with fatigue ($r=0.192$, $p=0.09$). In the multivariate model, none of the CSF parameters correlated with fatigue, neither in the discovery nor in the CSF validation cohort.

Discussion

In this study, we investigated the association of fatigue with clinical, brain MRI and CSF parameters in early MS.

Prevalence of fatigue in our cohort was in line with previous results in early MS patients from German MS Registry.[29] Although fatigue can be observed in the absence of any other MS symptom,[3] it increases with disability[30–33] and disease duration,[2,29,34] which was confirmed by our data. Furthermore, we found a robust association of fatigue and BDI, which is the recommended screening tool for depression in MS patients.[35] However, several BDI items overlap with fatigue symptoms (loss of energy, fatigue or difficulties in concentration) or are commonly observed in MS patients (sleep disorders, changes in appetite and sexual interest, irritability and emotional lability)[36,37] whether as symptom of the disease or adverse drug reaction and it might be impossible to entirely disentangle fatigue and depression in MS patients. This in turn means that MS patients are likely to achieve BDI scores, indicating minimal/mild depression and excluding these patients from the analysis might introduce a bias in the results. This might not apply for MS patients with higher BDI scores, indicating

clinically significant depression, which seems to resemble major depression.[38] Therefore, we repeated the analysis after exclusion of these patients without a meaningful change in the results.

We found weak correlations of fatigue with total and cortical grey matter that did not remain significant in the multivariate model that included EDSS score. We concluded that these correlations may not be specific for fatigue but rather reflect a longer or more severe disease course. Alternatively, the inclusion of an additional confounder might have decreased statistical power. In this case, however, one would expect consistent results among previous studies,[5] which is not the case. To our knowledge, only few studies investigating the association of fatigue with MRI measures controlled for EDSS or disease duration. Damasceno and coworkers have described significant association of fatigue with lower volumes of caudate and accumbens nucleus in a cohort of 49 MS patients.[33] Of note, *p* values became moderate (0.048, 0.047) after correction for EDSS. We did not confirm these associations in our cohorts, which were both larger (68 and 233 patients). Yet we acknowledge that our patients had a shorter disease duration (mean disease duration = 4.3/5.0 vs 6.2 years) and were less disabled (median EDSS = 1.0 and 1.5 vs 2.0).

Alternatively, fatigue in MS might be a consequence of chronic CNS inflammation. However, studies investigating different laboratory markers as potential biomarkers of fatigue in MS patients are inconclusive.[5] It is believed that inflammatory changes in the CNS parenchyma are reflected to a certain extent in the CSF compartment. Thus, we measured inflammatory parameters and immune cell subsets in the CSF compartment, but did not identify any significant association with the development of fatigue.

We acknowledge limitations of our study. A more detailed immune cell subtyping, for example, with memory and activation markers, might help to further investigate these contradictory findings. In our study, this was not possible as it was a post hoc analysis. With regard to MRI parameters, the investigation of diffuse WM changes and spinal cord pathology could add valuable information. Functional imaging may help to understand fatigue-related dysfunction at the network level, although changes identified through this technique are in principle compatible with either subtle structural damage or functional changes merely resulting from the inflammatory milieu. In a recent combined structural and functional MRI study, functional connectivity of basal ganglia correlated with fatigue severity in the absence of structural changes. This supports the hypothesis of a rather functional than structural cause of fatigue, at least in early MS.[39] Finally, we did not control for sleep disorders which might also contribute to fatigue in MS.[40]

In summary, we found a robust relation of fatigue with disease severity. We could link fatigue neither to damage of certain brain regions nor to a certain type of inflammatory activity. This, however, does not prove that those relations do not exist. Rather, demonstration of such associations may necessitate more sophisticated laboratory or MRI methods. Still, coincidence with disease duration and severity must be accounted for. Gaining a deeper understanding of fatigue may be very challenging as different aspects of MS pathology, as well as the premorbid personality, may eventually determine the final common pathway of fatigue.

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Declaration of Conflicting Interests

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References

1. Runia TF, Jafari N, Siepman DA, et al. Fatigue at time of CIS is an independent predictor of a subsequent diagnosis of multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2015; 86: 543–546.
2. Kister I, Bacon TE, Chamot E, et al. Natural history of multiple sclerosis symptoms. *Int J MS Care* 2013; 15: 146–158.
3. Lebrun C, Cohen M and Clavelou P. Evaluation of quality of life and fatigue in radiologically isolated syndrome. *Rev Neurol* 2016; 172: 392–395.
4. Cavallari M, Palotai M, Glanz BI, et al. Fatigue predicts disease worsening in relapsing-remitting multiple sclerosis patients. *Mult Scler* 2016; 22: 1841–1849.
5. Patejdl R, Penner IK, Noack TK, et al. Multiple sclerosis and fatigue: A review on the contribution of inflammation and immune-mediated neurodegeneration. *Autoimmun Rev* 2016; 15: 210–220.
6. Bol Y, Duits AA, Lousberg R, et al. Fatigue and physical disability in patients with multiple sclerosis: A structural equation modeling approach. *J Behav Med* 2010; 33: 355–363.
7. Morris G, Berk M, Galecki P, et al. The emerging role of autoimmunity in myalgic encephalomyelitis/chronic fatigue syndrome (ME/cfs). *Mol Neurobiol* 2014; 49: 741–756.
8. Bradley AS, Ford B and Bansal AS. Altered functional B cell subset populations in patients with chronic fatigue syndrome compared to healthy controls. *Clin Exp Immunol* 2013; 172: 73–80.
9. Maes M, Bosmans E and Kubera M. Increased expression of activation antigens on CD8+ T lymphocytes in Myalgic Encephalomyelitis/chronic fatigue syndrome: Inverse associations with lowered CD19+ expression and CD4+/CD8+ ratio, but no associations with (auto)immune, leaky gut, oxidative and nitrosative stress biomarkers. *Neuro Endocrinol Lett* 2015; 36: 439–446.
10. Brenu EW, Broadley S, Nguyen T, et al. A preliminary comparative assessment of the role of CD8+ T Cells in chronic fatigue syndrome/myalgic encephalomyelitis and multiple sclerosis. *J Immunol Res* 2016; 2016: 9064529.
11. Fluge O, Bruland O, Risa K, et al. Benefit from B-lymphocyte depletion using the anti-CD20 antibody rituximab in chronic fatigue syndrome. A double-blind and placebo-controlled study. *PLoS ONE* 2011; 6: e26358.
12. Dass S, Bowman SJ, Vital EM, et al. Reduction of fatigue in Sjogren syndrome with rituximab: Results of a randomised, double-blind, placebo-controlled pilot study. *Ann Rheum Dis* 2008; 67: 1541–1544.
13. De Flon P, Laurell K, Soderstrom L, et al. Improved treatment satisfaction after switching therapy to rituximab in relapsing-remitting MS. *Mult Scler*; 23: 1249–1257.
14. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011; 69: 292–302.
15. Penner IK, Raselli C, Stocklin M, et al. The Fatigue Scale for Motor and Cognitive Functions (FSMC): Validation of a new instrument to assess multiple sclerosis-related fatigue. *Mult Scler* 2009; 15: 1509–1517.
16. Beck AT, Steer RA and Brown GK. *Manual for the Beck Depression Inventory-II*. San Antonio, TX: Psychological Corporation, 1996.
17. Marrie RA, Cohen J, Stuve O, et al. A systematic review of the incidence and prevalence of comorbidity in multiple sclerosis: Overview. *Mult Scler* 2015; 21: 263–281.
18. Cepok S, Rosche B, Grummel V, et al. Short-lived plasma blasts are the main B cell effector subset during the course of multiple sclerosis. *Brain* 2005; 128: 1667–1676.
19. Shattuck DW, Mirza M, Adisetiyo V, et al. Construction of a 3D probabilistic atlas of human cortical structures. *Neuroimage* 2008; 39: 1064–1080.
20. Chard DT, Jackson JS, Miller DH, et al. Reducing the impact of white matter lesions on automated measures of brain gray and white matter volumes. *J Magn Reson Imaging* 2010; 32: 223–228.
21. Bezzola L, Merillat S, Gaser C, et al. Training-induced neural plasticity in golf novices. *J Neurosci* 2011; 31: 12444–12448.

22. Treadway MT, Waskom ML, Dillon DG, et al. Illness progression, recent stress, and morphometry of hippocampal subfields and medial prefrontal cortex in major depression. *Biol Psychiatry* 2015; 77: 285–294.

23. Muhlau M, Buck D, Forschler A, et al. White-matter lesions drive deep gray-matter atrophy in early multiple sclerosis: Support from structural MRI. *Mult Scler* 2013; 19: 1485–1492.

24. Keihaninejad S, Heckemann RA, Fagiolo G, et al. A robust method to estimate the intracranial volume across MRI field strengths (1.5T and 3T). *Neuroimage* 2010; 50: 1427–1437.

25. Good CD, Johnsrude IS, Ashburner J, et al. A voxel-based morphometric study of ageing in 465 normal adult human brains. *Neuroimage* 2001; 14: 21–36.

26. Geurts JJG, Calabrese M, Fisher E, et al. Measurement and clinical effect of grey matter pathology in multiple sclerosis. *Lancet Neurol* 2012; 11: 1082–1092.

27. Henry RG, Shieh M, Amirbekian B, et al. Connecting white matter injury and thalamic atrophy in clinically isolated syndromes. *J Neurol Sci* 2009; 282: 61–66.

28. R-Core-Team. *R: A language and environment for statistical computing, version 3.2.4*. Vienna: R Foundation for Statistical Computing, 2016.

29. Stuke K, Flachenecker P, Zettl UK, et al. Symptomatology of MS: Results from the German MS Registry. *J Neurol* 2009; 256: 1932–1935.

30. Iriarte J, Subira ML and Castro P. Modalities of fatigue in multiple sclerosis: Correlation with clinical and biological factors. *Mult Scler* 2000; 6: 124–130.

31. Simpson S Jr., Tan H, Otahal P, et al. Anxiety, depression and fatigue at 5-year review following CNS demyelination. *Acta Neurol Scand* 2016; 134: 403–413.

32. Calabrese M, Rinaldi F, Grossi P, et al. Basal ganglia and frontal/parietal cortical atrophy is associated with fatigue in relapsing-remitting multiple sclerosis. *Mult Scler* 2010; 16: 1220–1228.

33. Damasceno A, Damasceno BP and Cendes F. Atrophy of reward-related striatal structures in fatigued MS patients is independent of physical disability. *Mult Scler* 2016; 22: 822–829.

34. Tedeschi G, Dinacci D, Lavorgna L, et al. Correlation between fatigue and brain atrophy and lesion load in multiple sclerosis patients independent of disability. *J Neurol Sci* 2007; 263: 15–19.

35. Goldman Consensus Group. The Goldman Consensus statement on depression in multiple sclerosis. *Mult Scler* 2005; 11: 328–337.

36. Figved N, Klevan G, Myhr KM, et al. Neuropsychiatric symptoms in patients with multiple sclerosis. *Acta Psychiat Scand* 2005; 112: 463–468.

37. Bamer AM, Johnson KL, Amtmann D, et al. Prevalence of sleep problems in individuals with multiple sclerosis. *Mult Scler* 2008; 14: 1127–1130.

38. Hasselmann H, Bellmann-Strobl J, Ricken R, et al. Characterizing the phenotype of multiple sclerosis-associated depression in comparison with idiopathic major depression. *Mult Scler* 2016; 22: 1476–1484.

39. Finke C, Schlichting J, Papazoglou S, et al. Altered basal ganglia functional connectivity in multiple sclerosis patients with fatigue. *Mult Scler* 2015; 21: 925–934.

40. Veauthier C and Paul F. Sleep disorders in multiple sclerosis and their relationship to fatigue. *Sleep Med* 2014; 15: 5–14.