Structural brain magnetic resonance imaging correlates of fatigue in patients with multiple sclerosis

PhD thesis

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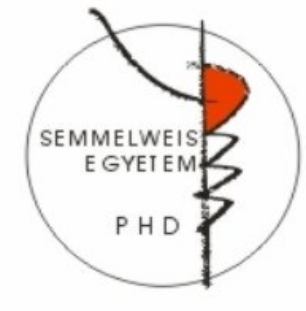

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List of Abbreviations

1F	1 time-point Fatigue
BPF	brain parenchymal fraction
CES-D	Center for Epidemiologic Studies Depression Scale
CLIMB	Comprehensive Longitudinal Investigations of MS at the Brigham and Women`s Hospital
CNS	central nervous system
DT	diffusion tensor
EDSS	Expanded Disability Status Scale
FA	fractional anisotropy
GM	gray matter
MFIS	Modified Fatigue Impact Scale
MRI	magnetic resonance imaging
MS	Multiple Sclerosis
NF	Never Fatigued
RF	Reversible Fatigue
RRMS	Relapsing-Remitting Multiple Sclerosis
SD	Standard Deviation
SF	Sustained Fatigue
SPMS	Secondary Progressive Multiple Sclerosis
T2LV	total T2 lesion volume
TBSS	tract-based spatial statistics
WB	whole brain
WM	white matter

1. INTRODUCTION

Multiple sclerosis (MS) is an inflammatory, demyelinating disorder of the central nervous system (CNS), currently affecting nearly 750,000 people in the United States [1]. In Hungary, the crude prevalence of MS was 130.8/100,000, and the crude incidence of MS was 5.4/100,000 in 2015 [2].

Fatigue is one of the most disabling symptoms in MS. It affects over 65% of MS patients, and 15-40% of these patients describe fatigue as their most severe symptom [3]. In general, fatigue is defined as an overwhelming sense of tiredness, lack of energy or feeling of exhaustion [4]. The Multiple Sclerosis Council for Clinical Practice Guideline defined MS-related fatigue as "a subjective lack of physical and/or mental energy that is perceived by the individual or caregiver to interfere with usual and desired activities"[5].

In neurologic diseases, fatigue can be differentiated based on its "peripheral" or "central" origin [6]. Peripheral fatigue (also referred to as muscle fatigability or performance fatigability) is induced by physical activity and disappears after rest [4, 6, 7]. It is associated with disorders of the neuromuscular junction or muscles, including myasthenia gravis, polymyositis and muscular dystrophies [6]. Central fatigue is a subjective sensation that is mediated by the CNS [6]. In contrast to peripheral fatigue, central fatigue is frequently present at rest [4]. Central fatigue has been associated with several diseases affecting the CNS, including Parkinson`s disease, stroke and narcolepsy [6]. MS patients experience both peripheral fatigue and central fatigue. A recently published meta-analysis study showed a moderate significant correlation ($r=0.31$, $p<0.001$) between these symptoms in MS, however, the overlap between their pathogenesis has not been clarified [7].

MS-related fatigue differs from fatigue experienced by persons without MS. MS-related fatigue (1) is not the same as muscle weakness, (2) it affects not only motor but also cognitive performance, causing a sense of memory impairment and difficulty maintaining concentration, (3) usually hampers daily basic functions, (4) may develop spontaneously at any time, (5) may worsen as the day progresses, (6) can be triggered by mental or physical activity, heat, humidity, acute infection and food ingestion, (7) can be alleviated by cooler temperature, daytime sleep or rest without sleep [3, 5, 6, 8].

Our recent work assessed the predictive value of fatigue toward conversion to confirmed moderate – severe disability in patients with Relapsing-Remitting MS (RRMS)

[9]. The results of that study showed that fatigue (measured by the Modified Fatigue Impact Scale (MFIS)) was significantly higher in converter versus non-converter MS patients suggesting that fatigue impact is a promising indicator of risk for disease worsening in RRMS patients.

Despite its clinical significance, the etiology and pathophysiology of MS-related fatigue is not well understood. Neural, immune, endocrine and metabolic mechanisms have all been proposed [5, 10, 11]. In addition, several other confounding factors, such as co-morbid depression, anxiety, and sleep abnormalities, altered reward responsiveness, as well as physical activity and medications, may interfere with the perceived level of fatigue [11, 12].

We recently conducted a detailed literature review on magnetic resonance imaging (MRI) correlates of MS-related fatigue to (1) summarize consistent findings regarding brain circuitry associated with fatigue in MS, (2) contextualize these findings with the neurochemistry of the relevant circuits, and (3) discuss future perspectives with regards to impact on fatigue management of MS patients and methodological challenges towards improved understanding of fatigue pathogenesis [13]. Among the plethora of neuroimaging studies investigating the relationship between structural brain abnormalities and fatigue in MS, several studies showed significant associations, but the replicability across these studies was limited, and they often provided inconsistent anatomical distribution patterns [13]. Several studies found no significant association between MS-related fatigue and structural brain damage [13]. The limited replicability of these studies is likely due, at least in part, to the criteria applied to classify patients. Previous neuroimaging studies allocated MS patients into “fatigued” or “non-fatigued” groups using a single time-point assessment of fatigue, which may not be adequate to summarize the fluctuating dynamics of this symptom [14]. A group allocation based on one single fatigue assessment can result in misclassifications that may lead to inconclusive results. In a recently completed study, our research group also tested the hypothesis that fatigue is associated with white matter (WM) lesion load in MS patients stratified into a “fatigued” or “non-fatigued” group based on a single time-point fatigue assessment [15]. The results of that study suggested that fatigue is not associated with global or tract-specific lesion load assessed in 19 WM tracts [15].

The overarching hypothesis of our studies described in this PhD thesis is that persistent fatigue is more likely to be caused by irreversible neurodegeneration, whereas fluctuating fatigue may reflect reversible pathobiological changes (e.g. inflammatory cytokine and hormone levels) not directly affecting brain morphology. Under this hypothesis, we proposed a novel classification that reflects temporal patterns from longitudinal fatigue impact assessments: Sustained Fatigue (SF: experienced clinically significant fatigue over the most recent two years), Reversible Fatigue (RF: did not report clinically significant fatigue at the most recent clinical visit, but did in the past), and Never Fatigued (NF).

Our **first aim** was to investigate whether the aforementioned novel group allocation that reflects temporal dynamics of fatigue (i.e., SF, RF and NF) improves our ability to detect fatigue-associated global structural brain abnormalities in MS. The **second aim** consists in comparing SF, RF and NF patients for regional structural brain abnormalities using voxel-based image statistics.

2. OBJECTIVES

Specific Aim 1: To investigate whether a novel group allocation that reflects temporal dynamics of fatigue improves our ability to detect fatigue-associated global structural brain abnormalities [16].

Hypothesis: Taking into account the persistence of fatigue impact – and thereby limiting the confounding effect of fatigue-inducing factors that are transient – improves our ability to discern structural brain abnormalities associated with fatigue impact.

Approach: MS patients were selected from the Quality Of Life (QOL) subset of our longitudinal cohort study of over 2000 MS patients, named Comprehensive Longitudinal Investigations of MS at the Brigham and Women's Hospital (CLIMB). The QOL subset ($n > 800$) undergoes annual MRI and neurological examination and biennial QOL assessments, including fatigue and depression measurements. The selected patients were stratified based on biennial fatigue impact assessments into the following groups: Sustained Fatigue (SF, fatigued at the latest ≥2 assessments), 1 time-point Fatigue (1F, fatigued at the most recent assessment, non-fatigued at the penultimate assessment and may or may not reported fatigue at previous assessments); Reversible Fatigue (RF, non-fatigued at the latest assessment, but reported fatigue previously); and Never Fatigued (NF). Brain parenchymal fraction (BPF) and total T2 lesion volume (T2LV) were compared between these groups, and between groups derived using a conventional, single time-point fatigued versus non-fatigued stratification. Fatigue impact was assessed using the MFIS.

Specific Aim 2: To compare SF, RF and NF patients for regional brain diffusion abnormalities [17].

Hypothesis: SF patients show more pronounced regional structural brain damage than RF and NF patients.

Approach: SF, RF and NF patients were compared using voxel-based image statistics on fractional anisotropy (FA) images. Fatigue impact was assessed using the MFIS.

3. RESULTS

3.1. RESULTS – SPECIFIC AIM 1

3.1.1. Demographic and clinical characteristics

Demographic and clinical variables did not show significant differences among the four fatigue impact groups, except for Expanded Disability Status Scale (EDSS), which was significantly higher in 1F patients compared to the other three groups, as well as MFIS score, which were significantly higher in SF patients (Table 1).

Table 1 [16]: Characteristics of the study participants at the time of MRI. Normally distributed variables are summarized as mean (SD), while non-normally distributed variables as median (IQR). Abbreviations: BPF = Brain Parenchymal Fraction; CES-D = Center for Epidemiologic Studies Depression Scale; EDSS = Expanded Disability Status Scale; ICV = Intracranial Cavity Volume; IQR = interquartile range; MFIS = Modified Fatigue Impact Scale; N/A = not applicable; NF = Never Fatigued; SD = Standard Deviation; SPMS = Secondary Progressive Multiple Sclerosis; SF = Sustained Fatigue; RRMS = Relapsing-Remitting Multiple Sclerosis; RF = Reversible Fatigue, T2LV = T2 lesion volume; 1F = 1 time-point Fatigue. *Medication was a dichotomous variable, which was 1 if a patient received anti-fatigue and/or anti-depressant and/or anxiolytic drugs, and was 0 if a patient received none of these drugs.

	SF	**1F**	**RF**	**NF**	**p-value**
Number of Subjects	29	15	31	54	N/A
Age (years, mean, SD)	49 (9)	49 (9)	50 (10)	51 (7)	0.15
Female Sex (n, %)	23 (80)	13 (87)	23 (74)	44 (81)	0.77
Non-White or Hispanic (n, %)	3 (10)	0 (0)	1 (3)	2 (4)	0.38
Disease Category (n, RRMS/SPMS)	25/4	11/4	27/4	50/4	0.25

Disease Duration (years, median, IQR)	17 (12-20)	17 (12-20)	14 (12-17)	14 (11-21)	0.67
EDSS (median, IQR)	2 (1.5-3)	2.5 (2-3.5)	1.5 (1-2.5)	1.5 (1-2)	0.004
Number of MFIS Assessments (median, IQR)	7 (5-8)	8 (7-9)	7 (6-10)	7 (6-7)	0.06
Time between all MFIS Assessments (years, median, IQR)	1.6 (1.2-1.8)	1.4 (1.2-1.6)	1.4 (1.3-1.6)	1.5 (1.2-1.7)	0.44
Time between latest two MFIS Assessments (years, median, IQR)	2 (2-3)	2 (2-3)	2 (2-2)	2 (2-3)	0.60
MFIS Total (median, IQR)	51 (44-54)	41 (39-50)	27 (11-29)	17 (8-26)	<0.001
CESD (median, IQR)	17 (9-24)	15 (9-17)	8 (3-14)	6 (2-9)	<0.001
Medication* (n, %)	24 (83)	2 (13)	20 (65)	23 (43)	<0.001
T2LV (mL, median, IQR)	5.2 (2.3-14.9)	3.5 (2.1-13.5)	3.4 (1.4-6.7)	2.2 (1.0-4.2)	0.01
BPF (%ICV, mean, SD)	82 (4)	81 (3)	82 (3)	83 (3)	0.15

There was no significant difference in age and sex between the pooled SF+1F+RF+NF cohort comparted to the CLIMB cohort (Table 2). However, disease duration, EDSS and RRMS to Secondary Progressive MS (SPMS) ratio were significantly higher in the pooled SF+RF+NF cohort, while the non-white or Hispanic to white or non-Hispanic ratio was significantly higher in the CLIMB cohort (Table 2).

Table 2 [16]: Comparison of demographic and clinical variables between Sustained, 1-time, Reversible and Never Fatigued MS patients at the time of brain MRI. The continuous variables showed non-normal distribution and are summarized as median (IQR). Abbreviations: CES-D = Center for Epidemiologic Studies Depression Scale; CLIMB = Comprehensive Longitudinal Investigations of MS at the Brigham and Women`s Hospital; EDSS = Expanded Disability Status Scale; IQR = interquartile range; MFIS = Modified Fatigue Impact Scale; MS = multiple sclerosis; N/A = not applicable; NF = Never Fatigued; SD = Standard Deviation; SPMS = Secondary Progressive MS; SF = Sustained Fatigue; RRMS = Relapsing-Remitting Multiple Sclerosis; RF = Reversible Fatigue, 1F = 1 time-point Fatigue. [1]Only RR and SP patients are shown from the CLIMB cohort.

	CLIMB	**All Patients (SF+1F+RF+NF)**	**p-value**
Number of Subjects	2421	127	N/A
Age (years, median, IQR)	50 (41-58)	51 (45-56)	0.24
Female Sex (%)	73	80	0.20
Non-White or Hispanic (%)	11	5	0.032
Disease Category (RRMS/SPMS, %)[1]	81/19	86/14	0.049
Disease Duration (years, median, IQR)	14 (9-19)	15 (12-20)	0.0004
EDSS (median, IQR)	1 (1-3.5)	1.8 (1-2.5)	0.004

3.1.2. Comparison of white matter lesion load and brain parenchymal fractional anisotropy between the fatigue groups

T2LV showed significant differences between the four groups in unadjusted analysis (p=0.012, Table 1). When controlling for age, sex, disease duration, EDSS and ICV, the difference in T2LV remained significant (p=0.002). Post-hoc analyses showed that T2LV of the SF (p=0.005) and RF (p=0.043) groups was significantly higher compared to the NF group (Table 3, Figure 1). There was no significant difference in the other contrasts (Table 3, Figure 1).

The median T2LV of the SF group was 35% higher compared to the 1F or RF groups, and 60% higher compared to the NF group (Table 1). There was only a 3% difference in the median T2LV between the 1F and RF groups, and these groups showed a 35% higher median T2LV compared to the NF group (Table 1). The two-group comparison showed significantly higher T2LV in fatigued versus non-fatigued patients (p=0.040, Table 2, Figure 1). The difference between the group medians was 35%. Neither the four-group, nor the two-group analysis showed significant difference in BPF (Tables 1 and 3, Figure 1).

Correction for medication and CES-D had no considerable effect on the above-mentioned results (results are reported in Tables 4 and 5).

Table 3 [16]: Global structural brain MRI measures in fatigue impact groups. The fatigue groups were compared using general linear models controlling for age, sex, disease duration, EDSS (and ICV in the analysis of log T2LV). According to the group allocation based on one MFIS assessment, the fatigued group corresponds to or Sustained Fatigue (SF) and 1 time-point Fatigue (1F) groups, while the Reversible (RF) and Never Fatigue (NF) groups are nested in the non-fatigued group. Abbreviations: BPF = Brain Parenchymal Fraction; CI = confidence interval; EDSS = Expanded Disability Status Scale; ICV = Intracranial Cavity Volume; Log = logarithmic-transformed; NF = Never Fatigued MS patients; Reversible Fatigue (RF); Sustained Fatigue (SF); T2 lesion volume (log T2LV); 1 time-point Fatigue (1F).

	Outcome	**BPF (%ICV)**	**Log T2LV (mL)**
fatigued (SF+1F) vs non-fatigued (RF+NF)	**ß (95% CI)**	0.01 (-0.02 to 0.01)	0.47 (0.05 to 0.89)
	p	0.256	***0.030***
	Cohen`s d	-0.29	0.50
SF versus NF	**ß (95% CI)**	-0.01 (-0.03 to 0.00)	0.74 (0.23 to 1.25)
	p	0.088	***0.005***
	Cohen`s d	-0.35	0.74
1F versus NF	**ß (95% CI)**	-0.01 (-0.03 to 0.01)	0.53 (-0.14 to 1.20)
	p	0.283	0.120
	Cohen`s d	-0.58	0.59
RF versus NF	**ß (95% CI)**	-0.01 (-0.03 to 0.00)	0.51 (0.02 to 1.00)
	p	0.070	***0.043***
	Cohen`s d	-0.38	0.48
SF versus 1F	**ß (95% CI)**	0.00 (-0.02 to 0.02)	0.21 (-0.50 to 0.92)
	p	0.818	0.555
	Cohen`s d	0.17	0.14
SF versus RF	**ß (95% CI)**	0.00 (-0.02 to 0.02)	2.23 (-0.33 to 0.79)
	p	0.980	0.418
	Cohen`s d	0.00	0.26
1F versus RF	**ß (95% CI)**	0.003 (-0.02 to 0.02)	0.02 (-0.69 to 0.72)
	p	0.802	0.958
	Cohen`s d	-0.20	0.11

Table 4 [16]: Medication adjusted differences in MRI measures in fatigue impact groups. The fatigue groups were compared using general linear models controlling for age, sex, disease duration, EDSS, medication (and ICV in the analysis of log T2LV). According to the group allocation based on one MFIS assessment, the fatigued group corresponds to Sustained Fatigue (SF) and 1 time-point Fatigue (1F) groups, while the Reversible (RF) and Never Fatigue (NF) groups are nested in the non-fatigued group. Abbreviations: BPF = Brain Parenchymal Fraction; CI = confidence interval; EDSS = Expanded Disability Status Scale; ICV = Intracranial Cavity Volume; log T2LV = logarithmic-transformed T2 lesion volume.

	Outcome	**BPF (%ICV)**	**Log T2LV (mL)**
fatigued (SF+1F) vs non-fatigued (RF+NF)	**ß (95% CI)** **p**	-0.01 (-0.02 to 0.01) 0.323	0.45 (0.02 to 0.87) ***0.040***
SF vs NF	**ß (95% CI)** **p**	-0.01 (-0.03 to 0.01) 0.205	0.70 (0.15 to 1.25) ***0.013***
1F vs NF	**ß (95% CI)** **p**	-0.01 (-0.03 to 0.01) 0.218	0.55 (-0.13 to 1.24) 0.110
RF vs NF	**ß (95% CI)** **p**	-0.01 (-0.03 to 0.00) 0.113	0.49 (-0.02 to 0.99) ***0.058***
SF vs 1F	**ß (95% CI)** **p**	0.00 (-0.02 to 0.03) 0.854	0.15 (-0.63 to 0.92) 0.711
SF vs RF	**ß (95% CI)** **p**	0.00 (-0.02 to 0.02) 0.855	0.21 (-0.36 to 0.78) 0.466
1F vs RF	**ß (95% CI)** **p**	0.00 (-0.02 to 0.02) 0.959	0.07 (-0.67 to 0.81) 0.861

Table 5 [16]: Depression and medication adjusted differences in MRI measures in fatigue impact groups. The fatigue groups were compared using general linear models controlling for age, sex, disease duration, EDSS, medication, CES-D (and ICV in the analysis of log T2LV). According to the group allocation based on one MFIS assessment, the fatigued group corresponds to Sustained Fatigue (SF) and 1 time-point Fatigue (1F) groups, while the Reversible (RF) and Never Fatigue (NF) groups are nested in the non-fatigued group. Abbreviations: Brain Parenchymal Fraction (BPF); Center for Epidemiologic Studies - Depression Scale (CES-D); confidence interval (CI); Expanded Disability Status Scale (EDSS); Intracranial Cavity Volume (ICV); logarithmic-transformed T2 lesion volume (log T2LV).

	Outcome	**BPF (%ICV)**	**Log T2LV (mL)**
fatigued (SF+1F) vs non-fatigued (RF+NF)	**ß (95% CI)** **p**	-0.01 (-0.02 to 0.01) 0.338	0.54 (0.04 to 1.04) ***0.033***
SF vs NF	**ß (95% CI)** **p**	-0.01 (-0.03 to 0.01) 0.182	0.85 (0.23 to 1.46) ***0.008***
1F vs NF	**ß (95% CI)** **p**	-0.01 (-0.04 to 0.01) 0.192	0.72 (-0.03 to 1.46) 0.061
RF vs NF	**ß (95% CI)** **p**	-0.01 (-0.03 to 0.00) 0.103	0.53 (0.02 to 1.04) ***0.042***
SF vs 1F	**ß (95% CI)** **p**	0.00 (-0.02 to 0.03) 0.841	0.13 (-0.65 to 0.91) 0.741
SF vs RF	**ß (95% CI)** **p**	0.00 (-0.02 to 0.02) 0.984	0.32 (-0.29 to 0.92) 0.302
1F vs RF	**ß (95% CI)** **p**	0.00 (-0.03 to 0.02) 0.853	0.19 (-0.59 to 0.96) 0.634

The statistical power in the unadjusted analysis of T2LV was 88% in the SF versus NF comparison, and 81% in the fatigued versus non-fatigue comparison. To detect the observed difference in T2LV at the two-sided p=0.05 level and 80% power, a sample size of 25 patients per group was needed in the SF versus NF comparison, and a sample size of 49 per group was needed in the fatigued versus non-fatigued comparison.

Cohen`s d effect size was larger for T2LV and BPF in the SF versus NF, and 1F versus NF contrasts, as well as for BPF in the RF versus NF contrast compared to the fatigued versus non-fatigued contrast (Table 3).

In Figure 1, we showed the temporal dynamics of MFIS in our study participants. We observed that none of the SF patients had MFIS scores≤19. This raised the motivation to investigate how many RF, NF and 1F patients have at least one MFIS score≤19. We found that 65% of RF patients, 89% of NF and 40% of 1F patients had at least one score in this range (i.e. MFIS≤19). Furthermore, we calculated what percentage of SF, RF and 1F patients have MFIS scores≥50 and found that 96% of SF patients, 26% of RF and 53% of 1F patients had at least one MFIS scores≥50 (NF patients had no MFIS≥38).

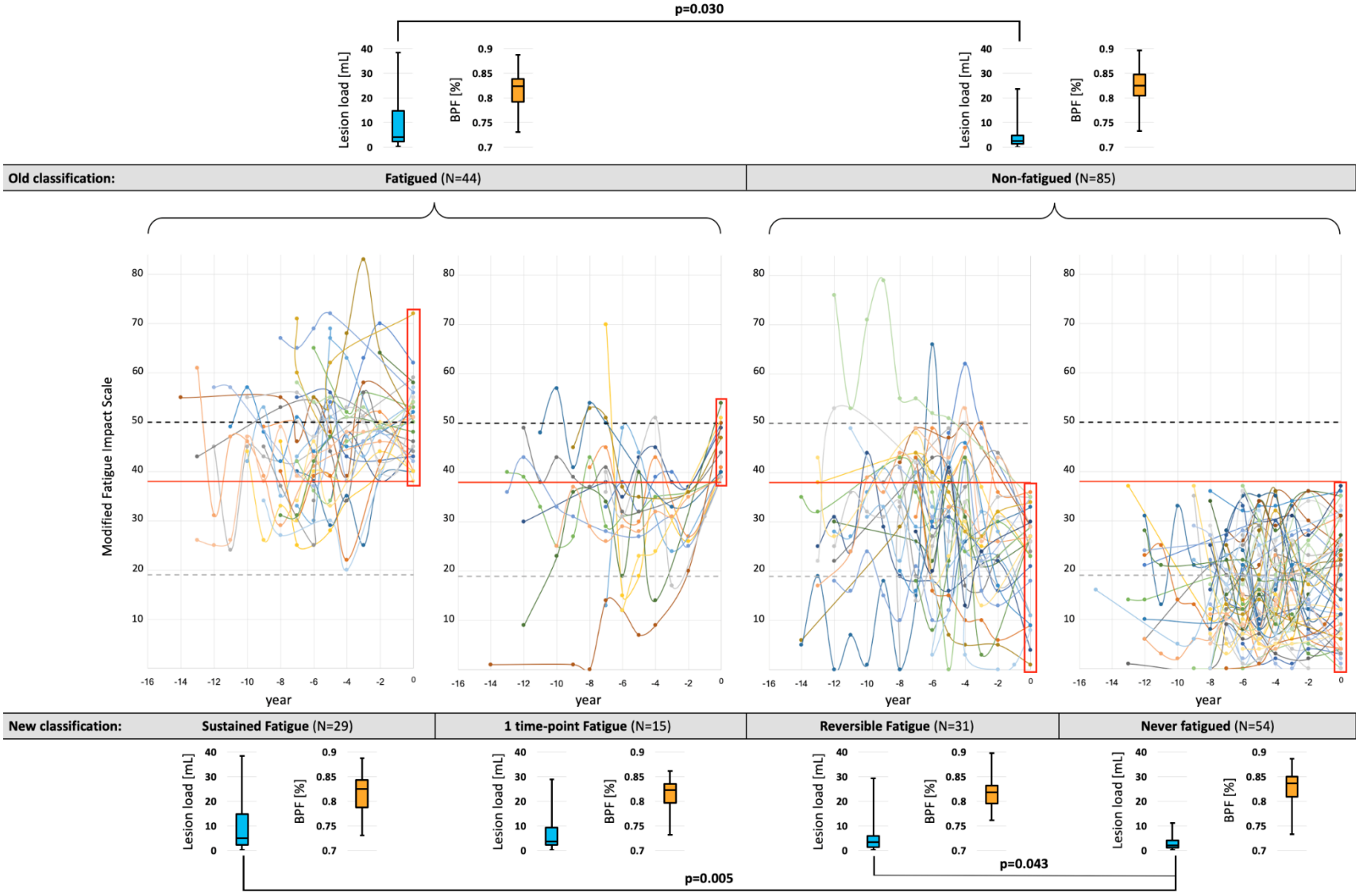
p=0.030
Lesion load [mL]
BPF [%]
Old classification:
Fatigued (N=44)
Non-fatigued (N=85)
Modified Fatigue Impact Scale
year
New classification:
Sustained Fatigue (N=29)
1 time-point Fatigue (N=15)
Reversible Fatigue (N=31)
Never fatigued (N=54)
p=0.005
p=0.043

Figure 1 [16]: Temporal evolution of fatigue measured using the Modified Fatigue Impact Scale (MFIS) as a function of time in patients with Sustained Fatigue, 1 time-point Fatigue, Reversible Fatigue as well as in Never Fatigued patients. The horizontal red line indicates the cut-off score for clinically significant fatigue (i.e., MFIS=38). Based on the most recent single time-point MFIS score (indicated by red rectangles at year 0), Sustained and 1 time-point Fatigue patients deemed "fatigued" (MFIS≥38), while Reversible and Never Fatigued patients deemed "non-fatigued" (MFIS<38). The horizontal dashed black line indicates MFIS=50, and the horizontal dashed grey line indicates MFIS=19. Box plots show lesion load and brain parenchymal fraction in each group.

3.2. RESULTS – SPECIFIC AIM 2

3.2.1. Demographic, clinical and global MRI characteristics

There was no significant difference between our study population and the CLIMB cohort in age, sex, Center for Epidemiologic Studies Depression Scale (CES-D), total and subscale scores of MFIS (Table 6). Disease duration (p=0.0001) and EDSS (p=0.017) were significantly higher in our study population compared to the CLIMB cohort as a whole (Table 6).

We found no significant difference between the SF, RF and NF groups in age, sex, disease duration, EDSS, and time between MFIS assessment and MRI scan (Table 6). CES-D, total and subscale MFIS scores from the latest assessment were significantly higher in the SF group compared to the RF and NF groups (p<0.001; Table 6). Those scores were not significantly different between RF and NF patients (Table 6).

Table 6 [17]: Comparison of demographic and clinical variables between Sustained, Reversible and Never Fatigued MS patients. Results are presented as mean (standard deviation). * $p<0.05$ versus CLIMB using Wilcoxon rank sum test. ** $p<0.05$ versus RF and NF using Kruskal-Wallis rank test followed by Dunn post-hoc test. Abbreviations: CES-D = Center for Epidemiologic Studies Depression Scale; EDSS = Expanded Disability Status Scale; MFIS = Modified Fatigue Impact Scale; MFIS-cog = cognitive subscale score of MFIS; MFIS-phys = physical subscale score of MFIS; MFIS-psych = psychosocial subscale score of MFIS; N/A = not applicable; NF = Never Fatigued patients; RF = Reversible Fatigue patients; RRMS = Relapsing-Remitting MS; SF = Sustained Fatigue patients; SPMS = Secondary Progressive MS.

	CLIMB (n=2421)	**SF+RF+NF (n=93)**	**SF (n=26)**	**RF (n=25)**	**NF (n=42)**
Age (years)	49.5 (12.3)	50.6 (8.5)	48.5 (9.2)	51.2 (10.0)	51.5 (7.0)
Gender (female/male %)	73/27	77/23	77/23	75/25	81/19
Disease duration (years)	13.7 (8.5)	16.9 (7.7)*	18.1 (6.9)	15.9 (7.6)	16.8 (8.2)
Disease category (RRMS/SPMS/other %)	71/17/12	89/11/0	85/15/0	90/10/0	93/7/0
Time between MFIS assessments and MRI scan (months)	N/A	5.7 (5.8)	5.34 (5.7)	4.9 (5.7)	6.5 (5.9)
EDSS	2.6 (2.3)	1.9 (1.4)*	2.3 (1.6)	2.1 (1.7)	1.6 (1.6)
MFIS-total	26.4 (17.9)	27.9 (17.4)	50.6 (8.0)**	22.4 (10.3)	8.5 (6.1)
MFIS-cog	11.9 (8.5)	13.4 (9.0)	24.3 (5.8)**	10.2 (5.6)	8.5 (6.1)
MFIS-phys	12.3 (9.0)	12.5 (8.5)	22.2 (4.7)**	10.7 (6.1)	7.6 (6.5)
MFIS-psych	2.2 (2.0)	2.2 (2.0)	4.1 (1.7)**	1.5 (1.3)	1.3 (1.6)

CES-D [number of patients with CES-D≥16]	10.3 (8.8) [177]	10.0 (8.0) [20]	16.9 (8.8)** [14]	9.3 (7.5) [5]	6.2 (4.2) [1]

In the pooled cohort of SF+RF+NF patients, total MFIS score showed significant correlation with CES-D ($p<0.0001$, rho=0.56) and EDSS ($p=0.007$, rho=0.28). CES-D and EDSS were not significantly correlated ($p=0.64$, rho=-0.05).

T2LV was significantly higher in SF compared to RF ($p=0.009$) or NF ($p<0.001$), but there was no difference between RF and NF patients. Most of the T2 lesions occurred in periventricular areas in all groups (Figure 2). There was no significant difference in BPF between the groups.

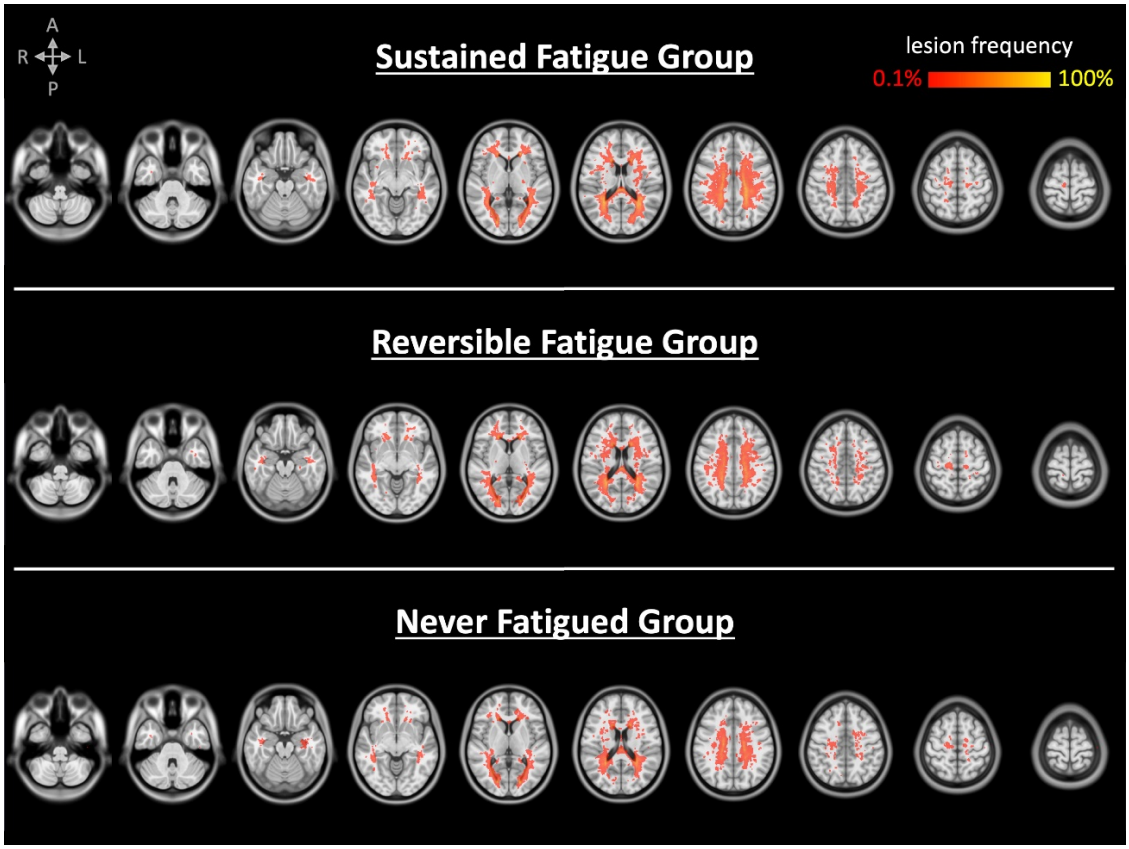

Figure 2 [17]: Lesion probability maps in the Sustained, Reversible and Never Fatigued groups. Voxel intensity represents the frequency of lesion occurrence in that voxel (ie, the probability of that voxel being a lesion).

3.2.2. Voxel-based comparison of fractional anisotropy between the fatigue groups

The whole brain (WB) diffusion tensor (DT) analysis adjusted for age, sex, disease duration, and EDSS showed significant differences between the groups in FA values in several bilateral brain regions, including cortical and WM areas of the frontal, temporal, parietal and occipital lobes, and subcortical structures, such as the striatum, thalamus, amygdala, hippocampus (Figures 3, 4, and 5). Changes in FA were tested in both directions (ie, negative and positive) in the following contrasts: SF versus NF, RF versus NF, SF versus RF. Our results showed only negative, and no positive associations, which were localized mainly in the WM. Some WM clusters appeared to extend into gray matter (GM) areas, lateral ventricles and perimesencephalic/peripontine cerebrospinal fluid. Although these changes may reflect GM abnormalities and brain atrophy, they may represent an artifact resulting from the Gaussian smoothing of DT images. In the SF versus NF contrast, the number of voxels with significantly lower FA was over 3-times higher compared to the SF versus RF contrast, and 6-times more compared to the RF versus NF contrast (Table 7).

Table 7 [17]: Pairwise comparison of MS patients with SF, RF and NF using voxel-based whole brain FA analysis controlling for age + sex + disease duration + EDSS ± total brain white matter lesion load ± CES-D. Abbreviations: CES-D = Center for Epidemiologic Studies – Depression Scale; DD = disease duration; EDSS = Expanded Disability Status Scale; FA = fractional anisotropy; N/A = not applicable; NF = Never Fatigued; p_{FWE} = family-wise error-corrected p value; RF = Reversible Fatigue; SF = Sustained Fatigue.

Pairwise group comparison	**Covariates in General Linear Models**	**Peak p_{FWE} value**	**Number of voxels with significantly lower FA**
SF versus NF	age+sex+DD+EDSS	<0.001	240,395.
	age+sex+DD+EDSS+lesion load	<0.001	21,875.
	age+sex+DD+EDSS+lesion load+CES-D	<0.001	10,022.
SF versus RF	age+sex+DD+EDSS	<0.001	71,517.
	age+sex+DD+EDSS+lesion load	0.954	N/A
	age+sex+DD+EDSS+lesion load+CES-D	0.290	N/A
RF versus NF	age+sex+DD+EDSS	<0.001	38,041.
	age+sex+DD+EDSS+lesion load	0.851	N/A
	age+sex+DD+EDSS+lesion load+CES-D	0.117	N/A

When controlling also for T2LV (in addition to age, sex, disease duration, and EDSS), the number of voxels with significantly lower FA decreased by a factor of 20 in the SF versus NF contrast, but there was no difference in SF versus RF or RF versus NF patients (Figures 3-5, Table 7). FA values in the following regions (presumably in the WM associated with them, given the reduction in FA) remained significantly lower in SF compared to NF patients: bilateral fronto-orbital and subgenual regions, right cingulate, right superior and middle temporal and temporal polar regions and right temporal WM, right insular and peri-insular area (including the external and extreme capsules and claustrum), bilateral fornix, body of corpus callosum, bilateral anterior limb of internal capsule, bilateral pre- and postcommisural striatum, left thalamus, right amygdala and hippocampal/parahippocampal region and right crus cerebri.

When controlling also for CES-D (in addition to age, sex, disease duration, EDSS, T2LV), the following regions showed significant association in SF versus NF (Figure 3): bilateral fronto-orbital and subgenual regions, right superior temporal and temporal polar regions and right temporal WM, right insular and peri-insular area (including the external and extreme capsules and claustrum), bilateral anterior limb of internal capsule, bilateral precommisural striatum, right amygdala and hippocampal/parahippocampal region and right crus cerebri. The SF versus RF and RF versus NF contrasts showed no significant association after controlling for CES-D (Figures 4 and 5).

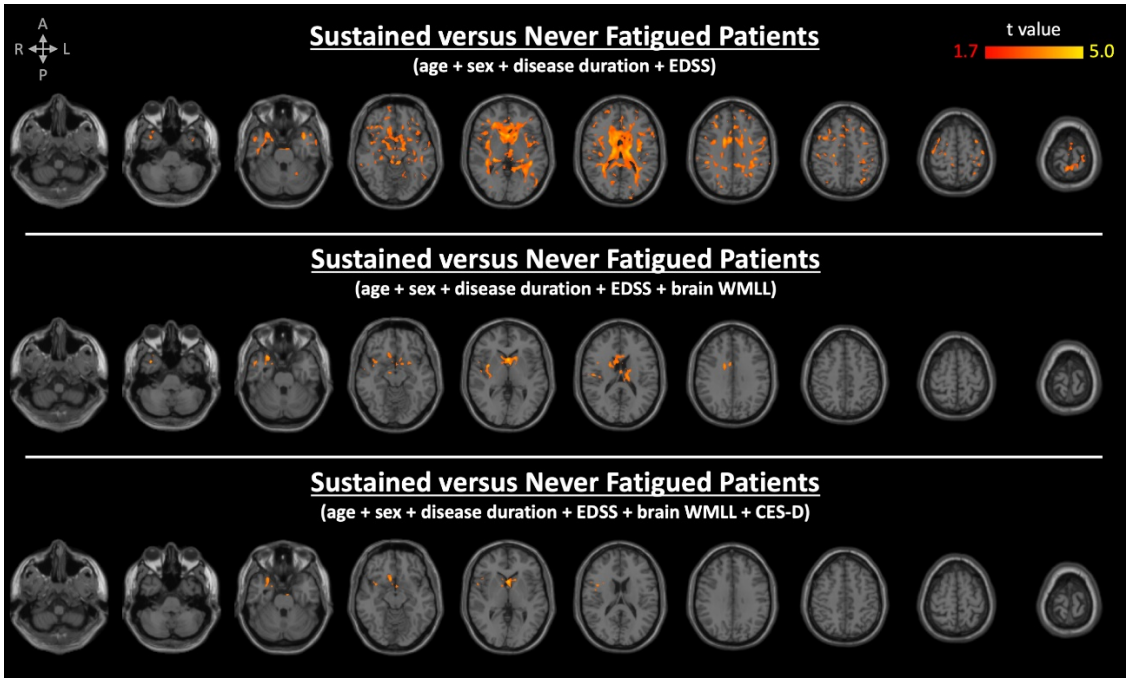

Figure 3 [17]: Brain areas with significantly lower fractional anisotropy in Sustained versus Never Fatigued patients when correcting for age + sex + disease duration + EDSS ± brain WMLL ± CES-D ± medication. Abbreviations: EDSS = Expanded Disability Status Scale, WMLL = T2 white matter lesion volume, CES-D = Center for Epidemiologic Studies Depression Scale.

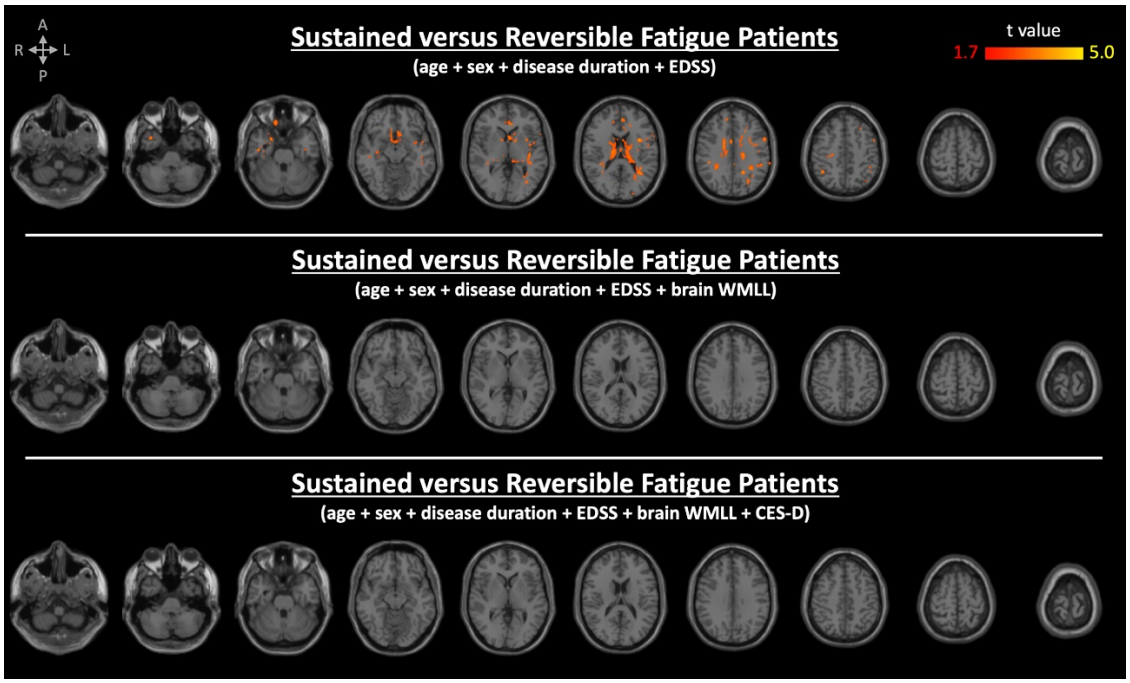

Figure 4 [17]: Brain areas with significantly lower fractional anisotropy in Sustained versus Reversible Fatigue patients when correcting for age + sex + disease duration + EDSS ± brain WMLL ± CES-D ± medication. Abbreviations: EDSS = Expanded Disability Status Scale, WMLL = T2 white matter lesion volume, CES-D = Center for Epidemiologic Studies Depression Scale.

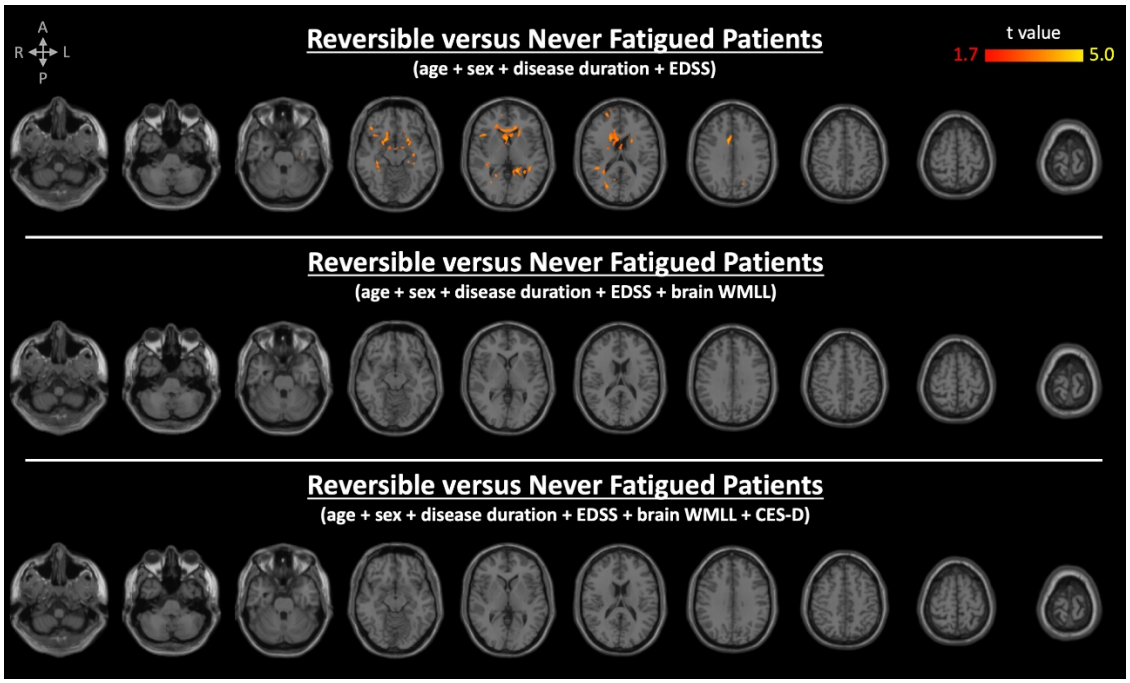

Figure 5 [17]: Brain areas with significantly lower fractional anisotropy in Reversible versus Never Fatigued patients when correcting for age + sex + disease duration + EDSS ± brain WMLL ± CES-D ± medication. Abbreviations: EDSS = Expanded Disability Status Scale, WMLL = T2 white matter lesion volume, CES-D = Center for Epidemiologic Studies Depression Scale.

4. DISCUSSION

Our first aim was to assess whether taking into account fatigue persistence can improve our ability to discern global structural brain abnormalities associated with fatigue. Therefore, we developed and tested a novel classification of fatigue based on retrospective longitudinal MFIS scores. The most salient finding of the aim 1 analysis is that discriminating patients by their longitudinal MFIS pattern improves the power to detect pathological MRI correlates of fatigue. Specifically, when confirming SF or NF status through more than one observation, we noted a significant increase in discriminating power.

Figure 1 shows that the MFIS trajectories observed in the studied patient population are consistent with the notion that fatigue is a fluctuating symptom of MS [14]. Our fatigue classification clearly differentiates four groups of patients with distinct temporal fatigue dynamics (i.e., SF, 1F, RF and NF). The primary groups of interest were the SF, RF and NF groups. The 1F group was included in the analysis to better represent the entirety of the traditional fatigued spectrum when combined with the SF group. SF patients experienced more severe fatigue not only at the most recent two MFIS assessments, but throughout the entire observed time-period compared to the other three groups. Even though both RF and NF patients were non-fatigued according to a traditional definition based on the most recent MFIS assessment (<38), RF patients had higher fatigue levels compared to NF patients during the observed time interval. These observations suggest that the cut-off of MFIS≥38 to define fatigued patients might not adequately reflect biologically relevant transitions in fatigue status, even though it is accepted as a clinically relevant threshold [18]. Our findings of long-standing differences in MFIS scores between the four groups warrant further scrutiny, with the aim of further discriminating biologically relevant patterns of fatigue that might inform our understanding and management of this debilitating symptom of MS.

Previous studies investigating the neural basis of fatigue deemed a patient fatigued based on one assessment only [13], thereby not accounting for fluctuations of fatigue [14]. Compared to a two-group classification based on one fatigue assessment, our four-group classification based on longitudinal assessments enables (1) to distinguish SF from 1F patients within fatigued patients (ie, MFIS≥38), and (2) to separate the non-fatigued spectrum (ie, MFIS<38) into two groups: RF and NF. Our results showed that

the observed difference in T2LV between the fatigued and non-fatigued groups was mainly driven by the difference between the SF and NF groups, while the 1F and RF groups were more similar to each other. In showing differences between the groups, our findings suggest that our classification is relevant for the study of the neural substrates of fatigue using quantitative MRI biomarkers. With a single fatigue assessment 1F patients would be deemed fatigued, while the RF patients would be deemed non-fatigued, with potential bias towards the null hypothesis. This may explain the null results of some of the previous studies that investigated the association between fatigue and lesion load and anatomical distribution in MS [13, 19-25].

Of note, the SF versus NF comparison was markedly better powered (ie, higher β coefficient and lower p value were observed under smaller sample size) and showed 48% larger effect size compared to the fatigued versus non-fatigued comparison. The sample size required under 80% power to detect significant ($p<0.05$) difference between the SF and NF groups was 49% lower compared to the sample size needed to detect significant difference between the fatigued and non-fatigued groups. Increasing the discriminating power of MRI studies by use of more stringent criteria leveraging longitudinal assessments of fatigue may also enable improved discrimination of regional differences in brain damage when using brain parcellation techniques or voxel-based analyses. This may resolve the inconsistencies in the scientific literature with regards to fatigue-associated anatomical patterns.

Our second aim was to compare SF, RF and NF patients for regional brain diffusion abnormalities. The most salient findings of the aim 2 analyses are the following: (1) both SF and RF are associated with diffuse structural brain damage. (2) SF patients have more widespread structural damage compared to RF. (3) Damage to the cingulo-postcommissural-striato-thalamic network are implicated in the development of both fatigue and depression, whereas damage to the ventromedial prefronto-precommissuro-striatal network and temporo-insular network were associated with fatigue independent of depression.

Only a few previous studies investigated the association of MS-related fatigue with regional brain diffusion abnormalities using voxel-wise approaches [20, 26-30]. These studies suggested the involvement of frontal networks in the pathogenesis of MS-related fatigue. Most of these studies used tract-based spatial statistics (TBSS) [20, 26-

29]. One TBSS study found association between fatigue and structural alterations in connections between frontal, temporal, parietal, occipital and deep WM areas [20]. The other TBSS studies failed to replicate these findings: two studies associated fatigue with lower FA only in a few tracts [26, 27], and two studies found no association at all [28, 29]. One study performed voxel-based FA analysis in the fronto-striato-thalamic WM and associated fatigue with lower deep frontal FA [30]. To overcome the anatomical restrictions of these studies, we performed an unbiased WB (supra- and infratentorial) voxel-based FA analysis. In line with the study of Bisecco et al [20], we observed lower FA in frontal, temporal, parietal, occipital and deep GM and WM areas in the SF versus NF, SF versus RF and RF versus NF patients, independent of age, sex disease duration and EDSS. These results suggest that – contrary to our hypothesis – both SF and RF are associated with structural brain damage. Nevertheless, the observed FA abnormalities were more widespread in SF compared to RF. For instance, cerebellar damage was seen only in SF patients. Taken together, the results of our voxel-based FA analyses suggest that persistent clinically relevant fatigue over years (ie, SF) is associated with more extensive brain WM damage than fluctuating fatigue (ie, RF). We found similar results when we compared SF, RF and NF patients for cortical and subcortical GM atrophy using voxel-based image statistics [31], and for mesocorticolimbic damage using diffusion tensor tractography [32].

Although conventional brain MRI is highly sensitive to macrostructural alterations (such as global and regional volume loss or detection of WM lesions), it lacks sensitivity to microscopic pathology involving normal-appearing WM and GM. These relatively more subtle changes can be detected using DT MRI. In the WM, FA is a scalar measure of fiber integrity and decrease in FA is suggestive of demyelination [33]. GM damage has been associated with increase in FA, possibly reflecting neuronal loss, swelling of neuronal cell bodies and/or reduced dendritic arborization [33, 34], although its histological correlates are still debated. In our study, voxel-based FA analysis was performed on the WB parenchyma including WM and GM.

MS lesions on conventional MRI are associated with lower FA values on DT images [33]. Most brain WM lesions were periventricular in our study (Figure 2). As expected, most of the observed FA differences were linked to macroscopic lesions. To address the anatomical specificity of our findings we added T2LV as a covariate in our

regression model. When we corrected also for T2LV, there was a 91% decrease in the number of voxels with significantly lower FA in SF versus NF. The remaining voxels were specifically clustered in frontal, temporal, insular, striatal and thalamic areas associated with SF. No significant difference was observed in SF versus RF and RF versus NF patients independent of T2LV suggesting that the use of longitudinal MFIS data to obtain more stringent groups of SF and NF patients increased our statistical power to identify specific anatomical regions associated with fatigue.

To further address the specificity of anatomical locations of WM damage towards fatigue not associated with depression, we added CES-D to our regression model. The relationship between fatigue and depression is not well understood in MS [11, 35, 36]. The clinical symptomatology and, accordingly, the clinical assessments/diagnosis of fatigue and depression show considerable overlap, which may raise the question whether these symptoms reflect separate entities with different etiology and pathophysiology or share similar pathogenesis and are part of the same spectrum, with fatigue at one end and depression at the other, with most individuals having some characteristics of both. Our results suggest that the association of fatigue with T2LV is independent of depression. However, correction for depression differentiated the following networks in SF versus NF patients. (1) The signal (ie, significantly lower FA) observed in orbito-frontal, subgenual, precommisural striatal, and temporal, insular and peri-insular regions survived correction for depression, suggesting that damage to the ventromedial prefronto-precommissuro-striatal network and the temporo-insular network may play a role in the development of fatigue independent of depression. (2) The signal observed in the cingulate, postcommissural striatum, fornical, callosal and thalamic areas disappeared upon correction for CES-D, suggesting that the cingulo-postcommissural-striato-thalamic network may play a role in the co-morbid development of both depression and fatigue. More signal was observed on the right side than on the left (eg, temporo-insular signal was observed only on the right side).

Our work demonstrated advantages of classifying patients according to temporal patterns of fatigue, when associating damage to select brain circuitries with fatigue in patients with MS [13, 14, 16, 17, 31, 37]. The detailed understanding of neurogenic mechanisms of fatigue in MS may enable improved characterization of fatigue phenotypes, especially in contrasting neurogenic to inflammatory and endocrine

mechanisms and unintended treatment side effects. While several drugs have demonstrated efficacy in improving wakefulness in other conditions (such as narcolepsy [38]), none have been proven effective in treating fatigue in MS [39, 40]. The ability to distinguish fatigue phenotypes might enable personalized management of this debilitating symptom.

While our studies highlighted the relevance of assessing temporal patterns of fatigue, retrospective data only included long interval (every 1-2 years) repeated measures of fatigue, which might not reflect pathophysiologically relevant patterns. Sustained Fatigue, as defined here, was useful in increasing statistical power of MRI comparisons, but is not intended as a biological or even clinical classification approach. It is quite possible that patients with MFIS≥38 at two time points two years apart, might experience low-fatigue levels in the intervening period. Further work is needed to investigate to what extent fatigue severity and fatigue consistency contribute to our findings to identify the most efficient and most discriminant temporal patterns, as well as to understand the biology underlying the perception and impact of fatigue. To this end, our research group recently developed a mobile application to enable circadian assessment of fatigue and other mood symptoms, with the longer-term goal to identify clinically and pathophysiologically relevant phenotypes of fatigue [41]. As an example of its potential significance, this mobile application will enable us to test the hypothesis that diverse fatigue phenotypes may respond to different mechanism-specific treatments. We hope that our work might pave the way for better predictors of fatigue treatment response, and identification of more selective treatment targets for fatigue management.

5. CONCLUSIONS

We developed and tested a novel classification of multiple sclerosis-related fatigue based on retrospective longitudinal Modified Fatigue Impact Scale scores. Our most salient findings are the following:

(1) Discriminating patients by their longitudinal Modified Fatigue Impact Scale pattern improves the power to detect pathological MRI correlates of fatigue. Specifically, when confirming Sustained Fatigue or Never Fatigued status through more than one observation, we noted a significant increase in discriminating power.

(2) Both Sustained Fatigue and Reversible Fatigue are associated with diffuse structural brain damage.

(3) Sustained Fatigue patients have more widespread structural brain damage compared to Reversible Fatigue.

(4) Damage to the cingulo-postcommissural-striato-thalamic network are implicated in the development of both fatigue and depression, whereas damage to the ventromedial prefronto-precommissuro-striatal network and temporo-insular network are associated with fatigue independent of depression.

6. SUMMARY

Background: The etiology of fatigue in multiple sclerosis (MS) is multifactorial. The neural basis of fatigue has been investigated by several magnetic resonance imaging (MRI) studies. The replicability of these studies was limited due, at least in part, to the criteria applied to classify patients. Previous neuroimaging studies allocated MS patients into "fatigued" or "non-fatigued" groups using a single time-point assessment of fatigue, which may not be adequate to summarize the fluctuating dynamics of fatigue.

Objectives: (1) To investigate whether a novel group allocation that reflects temporal dynamics of fatigue improves our ability to detect fatigue-associated global structural brain abnormalities. (2) To investigate the association of fatigue with regional structural brain damage, using the aforementioned group allocation strategy.

Methods: Patient stratification based on biennial fatigue assessments: Sustained Fatigue (SF, fatigued at latest ≥2 assessments), 1 time-point Fatigue (1F, fatigued at the latest, but non-fatigued at the penultimate assessment); Reversible Fatigue (RF, non-fatigued at the latest assessment, but reported fatigue previously); and Never Fatigued (NF). 3 Tesla brain MRI was used to compare brain parenchymal fraction (BPF) and total T2 lesion volume (T2LV) between these groups, and between groups derived using a conventional, single time-point fatigued versus non-fatigued stratification. SF, RF and NF patients were compared also for region brain diffusion abnormalities using voxel-based fractional anisotropy (FA) analysis.

Results: The SF versus NF stratification yielded improved power with respect to T2LV compared to the conventional fatigued versus non-fatigued stratification. We found no significant differences in BPF between the groups. SF and, to a lesser extent, RF patients showed significantly lower FA in multiple brain regions compared to NF patients. In ventromedial prefronto-precommissuro-striatal and temporo-insular areas, the differences in FA between SF and NF (but not between RF and NF or RF and SF) patients were independent of T2LV and depression.

Conclusions: Taking into account temporal fatigue dynamics increases the statistical power with respect to T2LV, and may improve characterization of brain pathological correlates of MS-related fatigue. Damage to ventromedial prefronto-precommissuro-striatal and temporo-insular pathways appears to be a specific substrate of Sustained Fatigue in MS.

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