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Fatigue scores correlate with other self-assessment data, but not with clinical and biomarker parameters, in CIS and RRMS

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ABSTRACT

Background: Fatigue is common in multiple sclerosis and is associated with reduced quality of life. This study aimed to assess the correlation between fatigue scores and data from other self-assessment questionnaires, neuropsychological tests and neuroimaging, as well as data on neuroimmunological markers in cerebrospinal fluid (CSF) and serum/plasma, in clinically isolated syndrome (CIS) and relapsing remitting MS (RRMS).

Methods: Modified fatigue impact scale (MFIS) scores were determined in 38 patients with newly diagnosed CIS or RRMS at baseline and after one year in a prospective longitudinal cohort study. Non-parametric correlation analyses were used to assess associations between MFIS scores and other self-assessment questionnaire data (Hospital Anxiety and Depression scale (HAD), Multiple Sclerosis Impact Scale 29 (MSIS-29) and Short Form 36 (SF-36)), as well as with neuropsychological test performances (*e.g.* Auditory Consonant Trigram Test (ACTT)), clinical parameters (*e.g.* disease duration and expanded disability status scale (EDSS)), magnetic resonance imaging (MRI) data (number of T2 lesions in brain MRI and total brain volume) and several neurodegenerative/neuroinflammatory markers in CSF and serum/plasma (IL-1β, IL-6, CXCL1, CXCL10, CXCL13, CCL-22 in plasma; neurofilament light chain (NFL) in serum; IL-6, CXCL1, CXCL10, CXCL13, CCL22, NFL and chitinase-3-like-1 (CHI3L1) in CSF. CSF and serum/plasma from 21 age- and sex-matched healthy controls were available for comparison.

Results: At both baseline and one-year follow-up, fatigue scores correlated significantly with HAD, MSIS-29 and SF-36 scores and ACTT performance (Spearman´s rho 0.45–0.78, all $p \leq 0.01$) but not with the other neuropsychological test results, disease duration, EDSS ratings, number of T2 lesions, total brain volume or neurodegenerative/neuroinflammatory markers, including neurofilament light chain levels in CSF and serum. In group comparisons, MFIS scores were similar in patients fulfilling no evidence of disease activity-3 (NEDA-3) ($n = 18$) and patients not fulfilling NEDA-3 ($n = 20$) during one year of follow-up ($p > 0.01$).

Conclusions: In this cohort of patients with newly diagnosed CIS and RRMS, fatigue scores were associated with mood, disease impact on daily life and quality of life as well as with alterations of attentive functions. Study results indicate that subjective fatigue scores are not well reflected by some commonly used and objectively measurable disease parameters like EDSS, T2 lesions and NFL levels.

1. Introduction

Multiple sclerosis (MS) is a chronic disease characterized by inflammation and degeneration of the central nervous system (CNS). Patients with MS often have focal neurological symptoms and signs, as well as more general symptoms like fatigue, cognitive impairment and depression. Fatigue can be described as “a subjective lack of physical and/or mental energy that is perceived by the individual or the caregiver to interfere with usual or desired activity”, although there is no universally accepted definition of the phenomenon (Arnett and

Abbreviations: ACTT, Auditory Consonant Trigram Test; CHI3L1, chitinase-3-like-1; D-KEFS, Delis–Kaplan Executive Function System; DMT, disease modifying treatment; FSS, fatigue severity scale; HAD, Hospital Anxiety and Depression scale; MFIS, modified fatigue impact scale; MSIS-29, Multiple Sclerosis Impact Scale 29; NEDA-3, no evidence of disease activity-3; NFL, neurofilament light chain; PASAT, Paced Auditory Serial Addition Test; SF-36, Short Form 36; TMT, Trail Making Test; VFT, Verbal Fluency Test

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Strober, 2011). Fatigue is reported as one of the worst symptoms by many MS patients and it can affect daily life (Flensner et al., 2003) and work productivity (Kobelt et al., 2017) negatively.

Fatigue can be theoretically classified as primary or secondary fatigue (Kos et al., 2008). Primary fatigue is related to centrally mediated processes of the MS disease, *e.g.* demyelination and axonal loss in the CNS and immunological factors, while secondary fatigue is related to other factors accompanying the MS illness, *e.g.* depression, sleep disturbances, reduced performance in daily life activities and side effects from medication (Kos et al., 2008). The total prevalence of fatigue varies depending on how it is defined and measured, as well as depending on patient sample (Rottoli et al., 2017). It has been reported to be 47% in patients with clinically isolated syndrome (CIS) (Runia et al., 2015) and 83% and 95%, respectively, in patients with MS (Kobelt et al., 2017; Minden et al., 2006). Due to the subjectivity of fatigue, self-report instruments are used to assess it. Of the two most commonly used fatigue scales, the modified fatigue impact scale (MFIS) (Guidelines, 1998) has been suggested to assess the impact on cognitive and psychosocial functioning better than the more physically focused fatigue severity scale (FSS) (Krupp et al., 1989; Tellez et al., 2005). The psychometric properties of MFIS and FSS have been compared and evaluated (Amtmann et al., 2012; Learmonth et al., 2013).

Relapses, new T2 lesions in magnetic resonance imaging (MRI) and disability progression are conventional signs of disease activity in relapsing remitting multiple sclerosis (RRMS), and disability progression is often assessed by repeated expanded disability status scale (EDSS) (Kurtzke, 1983) ratings. However, these parameters do not well reflect cognitive impairment and fatigue (Kobelt et al., 2017). The pathophysiology of fatigue is incompletely understood (Rottoli et al., 2017). The literature on the possible links between inflammation and fatigue in MS has recently been reviewed (Chalah and Ayache, 2018) and although IL-6 (Malekzadeh et al., 2015; Alvarenga-Filho et al., 2017; Brenner et al., 2018) has been implicated, its association with fatigue is not firmly established. The established MS biomarker neurofilament light chain (NFL) (Salzer et al., 2010; Mellergard et al., 2017; Hakansson et al., 2017; Hakansson et al., 2018; Novakova et al., 2017) and the suggested MS biomarkers CXCL1 (Hakansson et al., 2017; Hakansson et al., 2018; Burman et al., 2014), CXCL10 (Hakansson et al., 2017; Hakansson et al., 2018; Burman et al., 2014) and CXCL13 (Hakansson et al., 2017; Hakansson et al., 2018; Novakova et al., 2017; Khademi et al., 2011) have not been examined in relation to fatigue scores. Taken together, more information is needed on the relationship between fatigue and other aspects of MS, to better understand the nature of fatigue and to investigate if biomarkers of fatigue can be identified to aid in evaluation and treatment of this symptom.

This study aimed to assess correlations between MFIS scores and other self-assessed scores reflecting anxiety, depression and quality of life, as well as correlations between MFIS scores and neuropsychological test results, clinical data, MRI data and inflammatory and neurodegenerative parameters in CSF and serum/plasma, in a longitudinally followed-up cohort of patients with newly diagnosed CIS and RRMS.

2. Materials and methods

2.1. Patients and controls

Forty-one patients with CIS or RRMS were consecutively enrolled in a prospective longitudinal cohort study of CIS and newly diagnosed MS at the Department of Neurology, University Hospital of Linköping, Sweden. All patients fulfilled the revised McDonald criteria from 2010 (Polman et al., 2011) for CIS or RRMS. All patients underwent clinical neurological examination (including EDSS assessment) performed by the same neurologist (IH), blood and CSF sampling and MRI examination at baseline and at one year follow-up. Data based on this cohort have previously been reported regarding neuroinflammatory and neurodegenerative markers and their prognostic value (Hakansson et al., 2017; Hakansson et al., 2018). Three of 41 patients declined to participate in the neuropsychological evaluation and they were not included in the present study due to lack of neuropsychology and questionnaire data. Thus, 38 patients completed a neuropsychological evaluation and a battery of questionnaires at baseline and at one year follow-up and they were included in the present study. Patients received immunomodulatory treatment according to Swedish clinical practice during the study period, from 2009 to 2013. Patient characteristics are presented in Tables 1 and 2. For blood and CSF parameters, 21 age- and sex-matched healthy controls (HC) were recruited from healthy blood donors. Healthy controls were free from past and current neurological and autoimmune disease and their clinical neurological examinations were normal, as were routine findings in CSF (Table 1). No medication, except oral contraceptives, was allowed in healthy controls.

Table 1
Patient and healthy control characteristics at baseline.

Clinical and laboratory data	Patients $n = 38$	Healthy controls $n = 21$	p-value
Women/men (% women)	31/7 (82%)	17/4 (81%)	1.0
Age[a] (years)	31 (25–37)	32 (26–43)	0.4
Diagnosis (CIS/RRMS)	17/21	N/A	
Relapse within last 2 months (yes/no)	20/18	N/A	
Disease duration[a,b] (months)	4.8 (1.4–11.3)	N/A	
Disease duration[b] (number of subjects)		N/A	
0–1 months	8		
1.25–2 months	6		
2.25–6 months	9		
6.5–12 months	7		
13–24 months	3		
25–36 months	2		
37–120 months	3		
EDSS[a]	2 (1–2)	all 0	
EDSS (number of subjects)			
0	*5*		
1.0	*12*		
1.5–2.0	*12*		
2.5–3.0	*4*		
3.5–4.5	*5*		
CSF mononuclear cell count[a]	4.6×10^6/L (1.7–9.1)	2.2×10^6/L (1.0–2.8)	< 0.01
Albumin ratio[a]	4.9 (3.3–5.9)	4.5 (3.6–5.2)	0.5
IgG index[a]	0.7 (0.5–1.2)	0.5 (0.5–0.5)	< 0.001
IgG synthesis index[a]	1.4 (1.0–2.3)	0.9 (0.9–1.0)	< 0.001
Oligoclonal CSF IgG bands (pos/neg)	31/7	0/21	< 0.001

p-values from Chi-square tests for sex distribution and oligoclonal bands and from Mann–Whitney U tests for age and CSF data.

[a] Median and within brackets interquartile range.

[b] Disease duration refers to time from first symptom suggestive of demyelinating disease. N/A: not applicable.

Table 2
Patient diagnoses, relapse status and treatment status over time.

Clinical and laboratory data	Baseline	1 year
Number of subjects	38	38
Diagnosis (CIS/RRMS)	17/21	11/27
Relapse within last 2 months (yes/no)	20/18	4/34
Treatment (number of subjects)		
No DMT	38	15
Interferon-β 1b	0	17
Interferon-β 1a	0	1
Fingolimod	0	1
Natalizumab	0	4

DMT: disease modifying treatment.

2.2. Ethics statement and patient consent

The study was approved by The Regional Ethics Committee in Linköping and written informed consent was obtained from all participants.

2.3. Cerebrospinal fluid and serum/plasma sampling and analyses

Details about CSF sampling and routine analyses, as well as the multiplex bead array, the enzyme-linked immunosorbent assays and the single-molecule array (Simoa) method used to determine levels of the neuroinflammatory and neurodegenerative markers, are given in Supplementary material and have also been published previously (Hakansson et al., 2017; Hakansson et al., 2018). IL-6 analyses in CSF and plasma were performed specifically for this study and details about the multiplex bead array used are also given in Supplemental material.

2.4. Neuropsychological tests

Four neuropsychological tests (Strauss et al., 2006) were included in this study: (1) The Delis–Kaplan Executive Function System (D-KEFS) initial letter Verbal Fluency Test (VFT), where the total score from three trials with different letters was recorded, (2) the D-KEFS Trail Making Test (TMT) with number-letter switching, (3) the Paced Auditory Serial Addition Test (PASAT) with numbers presented every 2.4 s and (4) the Auditory Consonant Trigram Test (ACTT), where the total number of correct letters was recorded. VFT and TMT were included to assess mainly executive functions, whereas ACTT and PASAT were included to assess mainly cognitive processing speed, working memory and divided attention. More information on neuropsychological tests are given in Supplementary materials.

2.5. Questionnaires and clinical interview

The MFIS (Guidelines, 1998) is a 21-item questionnaire which measures the impact of fatigue on physical (9-items), psychosocial (2-items) and cognitive (10-items) domains over the past four-weeks. MFIS total score was used in this study. Three further questionnaires were used to examine self-assessed physical health and mental health: The Hospital Anxiety and Depression scale (HAD) (Honarmand and Feinstein, 2009), the Multiple Sclerosis Impact Scale (MSIS-29) (Hobart et al., 2001) and the Short Form 36 (SF-36). HAD was divided into an anxiety score (HAD-A) and a depression score (HAD-D), and MSIS-29 was divided into a physical (MSIS-29 physical) and a psychological (MSIS-29 psychological) score. From SF-36, the physical component summary score (SF-36 physical) and the mental component summary score (SF-36 mental), were calculated (Sullivan and Karlsson, 1994). Separately from completing the abovementioned questionnaires, at clinical visits it was recorded whether patients either spontaneously reported tiredness as a symptom to the treating neurologist, or answered affirmative when asked about the presence of this symptom.

2.6. Magnetic resonance imaging and post processing

All subjects were examined on a 1.5 T Philips Achieve MRI scanner (Philips Healthcare, Best, The Netherlands) using an 8 channel phased array head coil, as detailed in Supplementary material. Quantitative MRI images were acquired using QMAP sequence (Warntjes et al., 2008). All MRI examinations were thoroughly reviewed by the same neuroradiologist and data on *e.g.* T2 lesions were recorded. Brain volume was calculated as brain parenchymal fraction (BPF) using SyMRI® version 8.0 (SyntheticMR, Linköping, Sweden).

2.7. Statistical analyses

Statistical analyses were performed using SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). Since several data sets showed a non-Gaussian distribution, non-parametric bivariate correlation analysis (Spearman) was used for correlation analyses, the Mann–Whitney *U* test was used to compare two independent groups and the Wilcoxon matched-pairs signed rank test was used to compare patients at baseline and at one year follow-up. Chi-square test was used to compare the patient and control groups regarding sex distribution and presence or absence of oligoclonal bands. Because of multiple testing in correlation analyses, a stringent significance level of Spearman´s rho ≥ 0.4 and $p \leq 0.01$ was used. As an extra precaution related to multiple testing of correlations, Bonferroni post hoc testing was also performed in the questionnaire and neuropsychological domains of the study, where our positive findings clustered. A *p*-value of ≤ 0.05 was considered to be significant in Mann–Whitney *U* tests in group comparisons of sociodemographic and clinical parameters and in Wilcoxon matched-pairs signed rank tests, while $p \leq 0.01$ was considered to be significant in the multiple questionnaire, neuropsychological, IL-6 and disease activity group comparisons. All *p*-values were based on two-tailed statistical tests.

3. Results

3.1. MFIS scores and clinical parameters

Descriptive statistics on questionnaire scores are presented in Supplementary Table 1. There were no significant differences between baseline scores and one year follow-up scores, except for HAD-A, where baseline scores were significantly higher ($p = 0.001$).

MFIS did not correlate with age, time from first symptom or EDSS, at baseline or at one year follow-up (all Spearman´s rhos < 0.4 and $p > 0.01$). Also, MFIS did not differ significantly with regard to diagnosis (CIS/RRMS) or relapse status (in relapse/not in relapse), at baseline ($p = 0.68$ and 0.32, respectively) or at one year follow-up ($p = 0.86$ and 0.92, respectively). Women had higher MFIS scores at baseline ($p = 0.04$) and at follow-up ($p = 0.05$). At one year follow-up, interferon beta treated patients ($n = 18$) and untreated patients ($n = 15$) had similar MFIS, HAD-A, HAD-D, MSIS-29 physical, MSIS-29 psychological, SF-36 physical and SF-36 mental scores (all $p > 0.5$ in Mann–Whitney *U* tests).

MFIS scores were significantly higher in patients who either spontaneously reported tiredness as a symptom to the treating neurologist, or answered affirmative when asked about the presence of this symptom, than in patients who did not ($p \leq 0.001$ at baseline as well as at one year follow-up).

3.2. MFIS scores in relation to HAD, MSIS-29 and SF-36

MFIS correlated significantly with HAD-A, HAD-D, MSIS-29 physical, MSIS-29 psychological, SF-36 physical and SF-36 mental at baseline as well as at one year follow-up, as shown in Table 3.

Patients were also divided into median split groups of “MFIS above median” (MFIS AM) and “MFIS below median” (MFIS BM), based on MFIS medians of 21 at baseline and 13.5 at one year. Significant differences were noted between the MFIS AM and MFIS BM groups regarding HAD-D, MSIS-29 physical, MSIS-29 psychological, SF-36 physical and SF-36 mental at both baseline and one year follow-up (all $p \leq 0.01$), but not regarding HAD-A ($p = 0.02$ at baseline and $p = 0.04$ at one year), as illustrated in Fig. 1.

3.3. MFIS scores in relation to neuropsychological test results

MFIS scores correlated significantly with ACTT results at baseline (Spearman´s rho -0.49, $p \leq 0.01$) as well as at one year follow-up

Table 3
Correlations between MFIS and HAD-A, HAD-D, MSIS-29 physical, MSIS-29 psychological, SF-36 physical and SF-36 mental, at baseline and at one year follow-up.

	HAD-A	HAD-D	MSIS-29 physical	MSIS-29 psychological	SF-36 physical	SF-36 mental
MFIS, BL	0.45*	0.63**	0.74**	0.77**	−0.64**	−0.70**
MFIS, 1y	0.48*	0.55**	0.78**	0.78**	−0.54**	−0.57**

Correlation coefficients (Spearman´s rho) from bivariate non-parametric correlation analyses (Spearman) are shown.
MFIS: Modified Fatigue Impact Scale, HAD-A: Hospital Anxiety and Depression scale anxiety score, HAD-D: Hospital Anxiety and Depression scale depression score, MSIS-29 physical: Multiple Sclerosis Impact Scale physical score, MSIS-29 psychological: Multiple Sclerosis Impact Scale psychological score, SF-36 physical: Short Form 36 physical component summary score, SF-36 mental: Short Form 36 mental component summary score, BL: baseline, 1y: one year follow-up.
* $p \leq 0.01$, which means that the results pass Bonferroni correction for the sum of the ten tests in the questionnaire and neuropsychological domains of the study.
** $p \leq 0.001$, which means that the results pass Bonferroni correction for the sum of the ten tests in the questionnaire and neuropsychological domains of the study.

(Spearman´s rho −0.58, $p \leq 0.001$), whereas there were no significant correlations with VFT (Spearman´s rhos −0.33 and −0.31), TMT (Spearman´s rhos 0.12 and 0.32) and PASAT (Spearman´s rhos −0.32 and −0.25). The one year ACTT correlation passed Bonferroni correction for the sum of the ten tests in the questionnaire and neuropsychological domains of the study, whereas the baseline ACTT correlation did not.

A significant difference was noted between the MFIS AM and MFIS BM group regarding ACTT at one year follow-up ($p = 0.001$), but not at baseline ($p = 0.02$), as illustrated in Fig. 2. There were no significant differences between the groups regarding VFT, TMT or PASAT results, neither at baseline nor at one year follow-up (all $p > 0.01$) (Fig. 2).

3.4. MFIS scores in relation to neuroinflammatory and neurodegenerative markers

IL-6 in CSF from patients was significantly higher than in healthy controls at both baseline and one year follow-up (both $p \leq 0.01$), whereas plasma levels did not differ (both $p > 0.01$) (Fig. 3).

Descriptive statistics on IL-6 and several other neurodegenerative and neuroinflammatory markers in patients and HC are presented in Supplementary Table 2. The subjects in this study belongs to a somewhat bigger cohort ($n = 41$), from which data on these markers, except IL-6, have previously been published (Hakansson et al., 2017; Hakansson et al., 2018). Supplementary Table 2 presents data from the particular 38 patients and 21 healthy controls included in the present study. MFIS scores in the patient group did not correlate significantly with any of these markers (IL-1β, IL-6, CXCL1, CXCL10, CXCL13, CCL-22 in plasma; neurofilament light chain (NFL) in serum; IL-6, CXCL1, CXCL10, CXCL13, CCL22, NFL, chitinase-3-like-1 (CHI3L1) in CSF), neither at baseline nor at one year follow-up (all Spearman´s rhos < 0.30 and all $p > 0.10$).

3.5. MFIS scores in relation to MRI parameters

MFIS at baseline did not correlate with BPF ($p = 0.7$) or total number of T2 lesions in the brain ($p = 0.5$) at baseline. MFIS at one year follow-up did not correlate with BPF ($p = 0.6$) or total number of T2 lesions in the brain ($p = 0.2$) at one year follow-up, and also not with BPF decrease from baseline to one year ($p = 0.8$) or number of new T2 lesions in the brain from baseline to one year ($p = 0.2$).

3.6. Self-assessment questionnaire scores and neuropsychological test results in relation to disease activity during follow-up

Patients that showed no relapses, no brain MRI activity (no new or enlarging T2 lesions or Gadolinium-enhancing lesions) and no sustained disability worsening (EDSS progression) during follow-up were classified as showing "no evidence of disease activity" − 3 (NEDA-3), while patients with relapses, brain MRI activity or sustained disability worsening were classified as showing evidence of disease activity (EDA).There were no significant differences in baseline or one year MFIS, HAD-A, HAD-D, MSIS-29 physical, MSIS-29 psychological, SF-36 physical and SF-36 mental scores between patients fulfilling NEDA-3 ($n = 18$) and patients not fulfilling NEDA-3 ($n = 20$) during one year of follow-up (all $p > 0.01$). There were also no significant differences in baseline or one year VFT, TMT, PASAT and ACTT results (all $p > 0.01$) between NEDA-3 and EDA patients.

4. Discussion

In this study, we report significant and consistent correlations between MFIS scores and other self-reported questionnaire data as well as ACTT performance at both baseline and one year follow-up in a cohort of newly diagnosed CIS and RRMS patients. In contrast, MFIS scores showed no significant correlations with an extensive panel of conventional disease activity parameters, including clinical, neuroimaging, and neuroinflammatory/neurodegenerative markers.

The correlations between MFIS scores and HAD-A, HAD-D, MSIS-29 physical, MSIS-29 psychological, SF-36 physical and SF-36 mental scores, respectively, were in a similar range at baseline and at one year follow-up. Our results are in line with previous reports of fatigue correlating with depression and anxiety in CIS (Runia et al., 2015) and MS populations (Bakshi et al., 2000; Kroencke et al., 2000; Wood et al., 2013), as well as reports of fatigue correlating with MSIS-29 scores (Wood et al., 2013; Mills and Young, 2011) and SF-36 scores (Nourbakhsh et al., 2016; Fernandez-Munoz et al., 2015) in MS. However, these studies did not include the neuroinflammatory/neurodegenerative markers that we have evaluated in relation to fatigue scores and most of the previous studies did not include neuroimaging parameters. Reflecting on the correlations detected in our study, it is important to remember that no conclusions about causality can be drawn from them. Regarding for instance fatigue and anxiety/depression, theoretically one of them could be the cause of the other, although a bidirectional relationship is more likely. As for cognition in MS, slowed cognitive processing speed and episodic memory decline are the most common cognitive deficits, with additional difficulties in executive function, verbal fluency, and visuospatial analysis (Sumowski et al., 2018). Previous reports on associations between fatigue and cognitive dysfunction are inconsistent (Hanken et al., 2015). ACTT and PASAT were included in this study to assess mainly cognitive processing speed, working memory and divided attention, whereas VFT and TMT were included to assess mainly executive functions. It seems reasonable that an association could exist between fatigue scores and the psychometric properties that ACTT reflect, but the same could be said about the other tests as well. It has been stated that PASAT is a more stressful test than ACTT (Ozakbas et al., 2004), which could imply that PASAT results are more strongly influenced by stress not related to CIS/MS and therefore do not correlate as strong as ACTT results with MFIS scores. The symbol digit modalities test (SDMT), which is recommended for brief cognitive assessment in MS (Sumowski et al., 2018), was unfortunately not included in our test battery. This is a limitation and in future studies SDMT should be included.

Although several neuroinflammatory and neurodegenerative markers in CSF, including IL-6, were significantly higher in patients than in healthy controls, we found no significant correlations between these parameters and MFIS scores in the patient group. We also did not see an association between IL-6 in plasma and MFIS scores. Others have

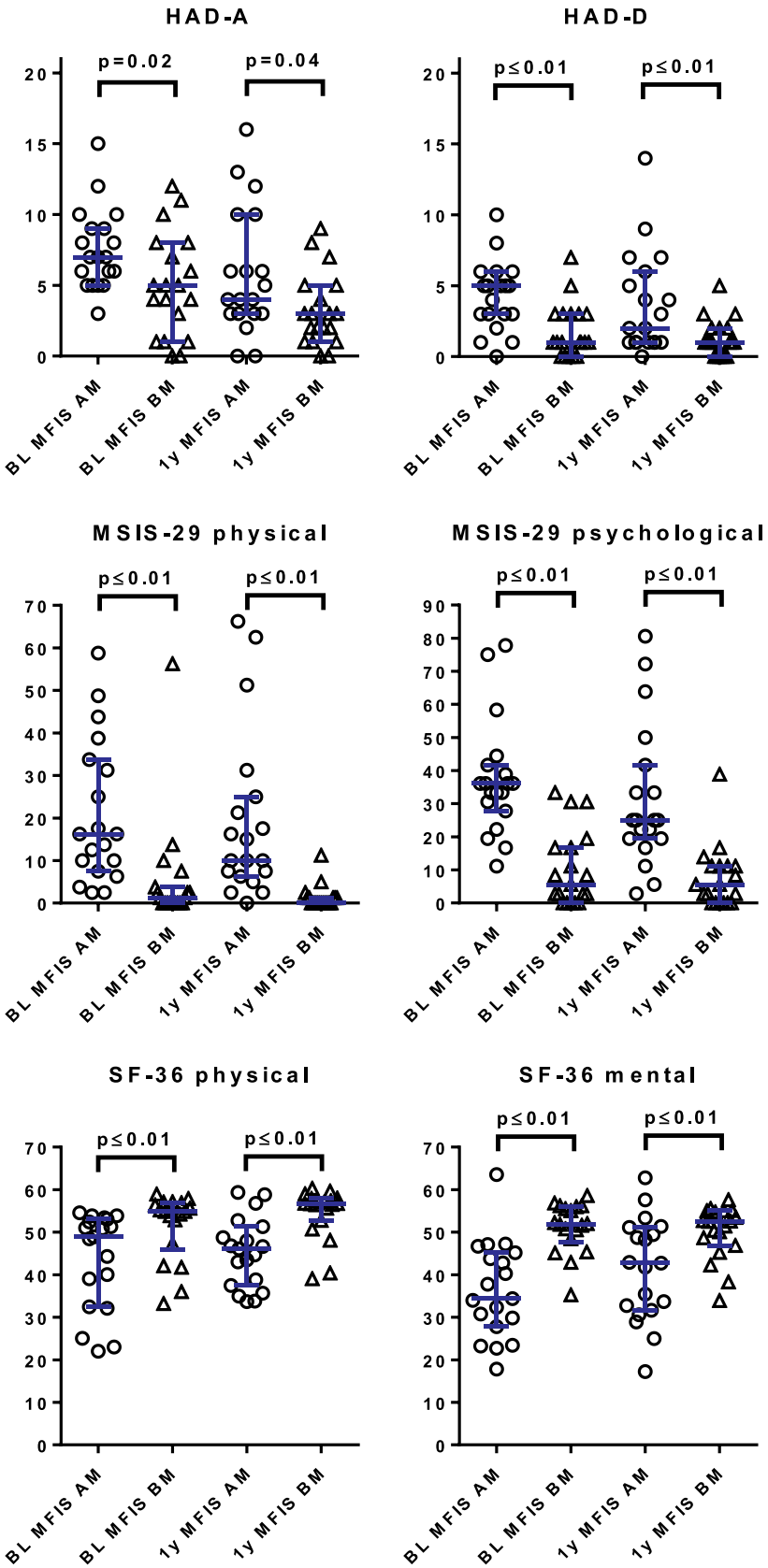

Fig. 1. Questionnaire scores in MFIS above median (MFIS AM) and MFIS below median (MFIS BM) groups at baseline (BL) and at one year follow-up (1y). *p*-values from Mann–Whitney *U* tests. MFIS: Modified Fatigue Impact Scale, HAD-A: Hospital Anxiety and Depression scale anxiety score, HAD-D: Hospital Anxiety and Depression scale depression score, MSIS-29 physical: Multiple Sclerosis Impact Scale physical score, MSIS-29 psychological: Multiple Sclerosis Impact Scale psychological score, SF-36 physical: Short Form 36 physical component summary score, SF-36 mental: Short Form 36 mental component summary score.

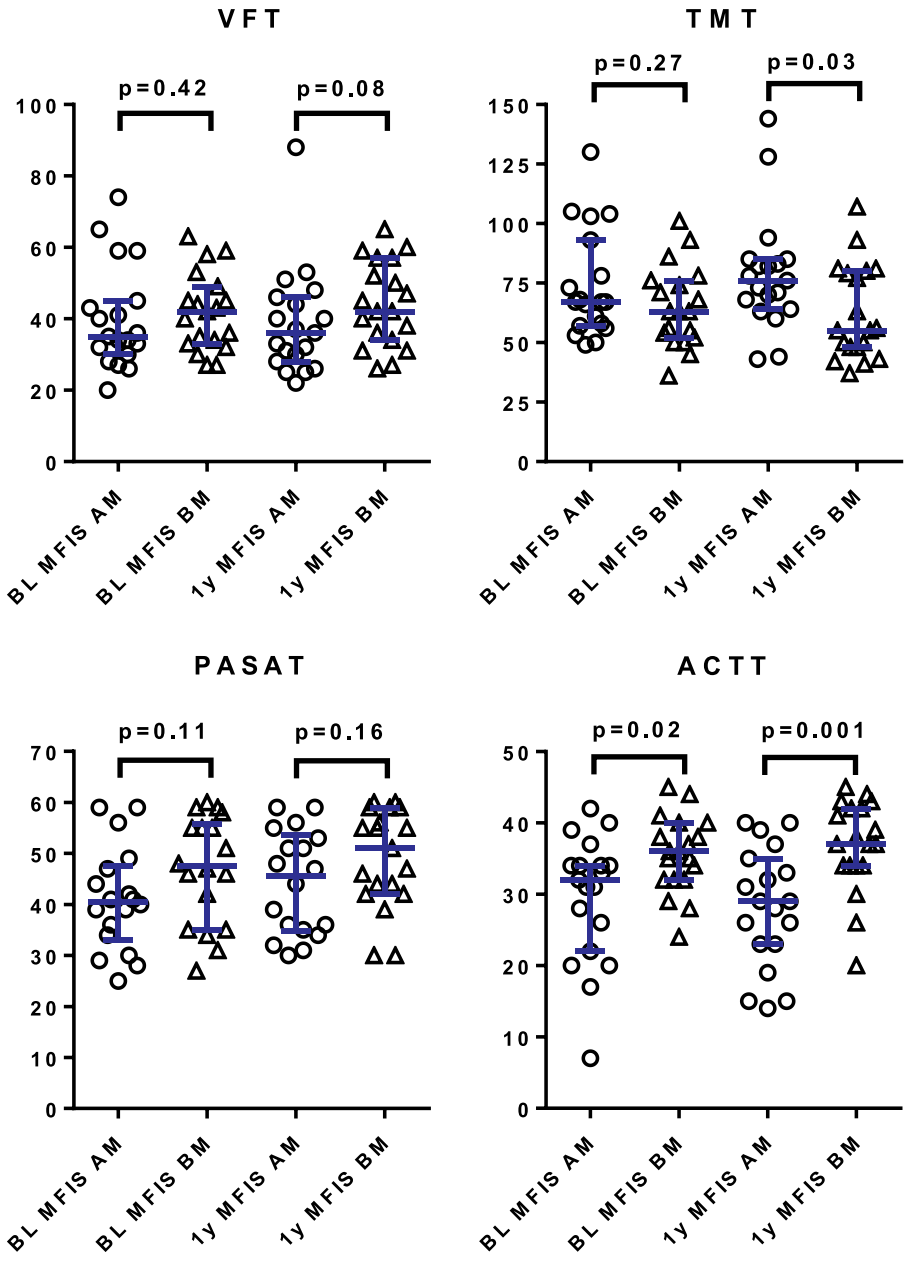

Fig. 2. Neuropsychological test results in MFIS above median (MFIS AM) and MFIS below median (MFIS BM) groups at baseline (BL) and at one year follow-up (1y). *p*-values from Mann–Whitney *U* tests. MFIS: Modified Fatigue Impact Scale, VFT: Verbal Fluency Test, TMT: Trail Making Test, PASAT: Paced Auditory Serial Addition Test, ACTT: Auditory Consonant Trigram Test.

reported that IL-6 in serum from MS patients was somewhat associated with fatigue scores in multiple regression analysis (Malekzadeh et al., 2015), but concentrations did not differ significantly between "fatigued" and "non-fatigued" patient groups in that study (Malekzadeh et al., 2015). Another study states that IL-6 levels in plasma were significantly higher among MS patients with fatigue than among patients without fatigue, with IL-6 levels also correlating with fatigue scores (Alvarenga-Filho et al., 2017). IL-6 levels in CSF were associated with self-rated depression and fatigue in patients with RRMS in a study that, interestingly, reported an MFIS median in the range of our median values (Brenner et al., 2018). IL-1β in serum did not differ between fatigued and non-fatigued patients in two studies (Malekzadeh et al., 2015; Akcali et al., 2017) and no associations were found between immune cell subsets in the CSF and fatigue (Biberacher et al., 2018). However, fatigue scores in relation to chemokines and NFL has not, to our knowledge, been evaluated before. We also did not detect any significant associations between MFIS scores and EDSS, BPF or number of T2 lesions. Some previous studies have found that fatigue and EDSS correlate (Biberacher et al., 2018; Simpson et al., 2016), while others have not, at least not after controlling for depression (Bakshi et al., 2000). Previous studies are inconsistent regarding fatigue in relation to brain volume and T2 lesions too, with associations between fatigue and brain volume/atrophy and/or T2 lesions reported by some (Tedeschi et al., 2007), but not by others (Runia et al., 2015), at least not when controlling for EDSS (Biberacher et al., 2018). However, whereas T2 lesion load and total brain volume usually have not been associated with fatigue, associations between fatigue and neuroimaging parameters like total and regional cortical volumes, thalamic damage and reduced functional connectivity in several regional areas have been proposed (Patejdl et al., 2016; Arm et al., 2019).

When comparing results from different studies, it is of importance to take into account factors that could explain inconsistencies in reported

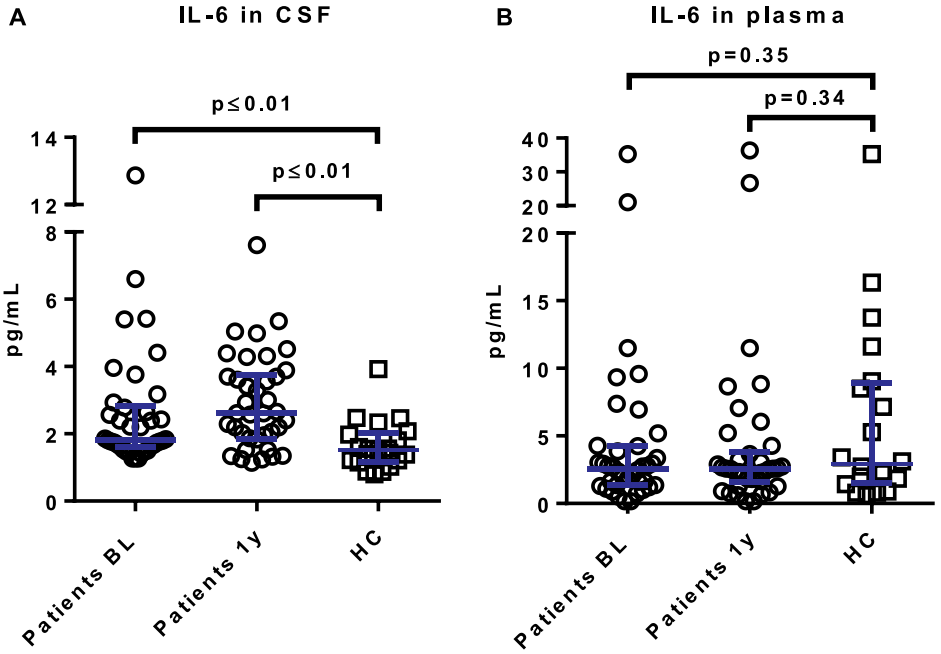

Fig. 3. Levels of IL-6 in CSF (A) and plasma (B) in healthy controls and in patients at baseline and at one year follow-up. Patients BL: Patients at baseline, Patients 1y: Patients at one year follow-up, HC: Healthy controls. *p*-values from Mann–Whitney *U* tests.

findings. Such factors include sample size, publication bias, differences in study populations regarding diagnoses, disease duration and EDSS scores, as well as methods used to assess and define the prevalence and level of fatigue. Strengths of this study are the broadly characterized patients (including extensive CSF data), the longitudinal design and the consistency of results at baseline and follow-up. A limitation of this study is the lack of MFIS, cognitive, emotional and behavioral assessments in healthy controls. Another limitation is the rather small cohort size and it could be argued that in a larger patient population significant correlations with clinical and biological parameters would have been found. We agree that this is possible, but we note that the associations with other questionnaire data were convincingly detected in our study and the cohort was also big enough to clearly demonstrate significant associations between *e.g.* baseline NFL levels and NEDA-3 during follow-up, as previously reported (Hakansson et al., 2017; Hakansson et al., 2018). Fatigue and MFIS scores can be influenced by other parameters than strict MS pathophysiology, such as family situation, work situation, cultural context and self-perceived performance in relation to own expectations as well as perceived expectations from others. Whereas neurofilament levels in an MS patient usually reflects axonal damage due to the MS disease alone, fatigue experience may be related to primary MS neuropathology, psychosocial secondary effects of the MS disease and/or non-MS related circumstances in life (medical co-morbidities as well as sociodemographic factors). This, in combination with the subjective nature of fatigue, makes it a complex and multifactorial phenomenon that may vary between individuals as well as within the same individual over time, despite perhaps the same/unchanged number, size and localization of T2 lesions in MRIs. Psychosocial secondary effects of the MS disease, non-MS related circumstances in life and non-MS related anxiety, depression or sleep disorders are factors which may have influenced our results and that we had no means of controlling for. We therefore want to stress that we cannot draw any conclusions about primary MS fatigue from this study. A higher proportion of patients in relapse and in stress at baseline than at follow-up, in combination with an impact of coping mechanisms at follow-up, may explain why HAD-A scores were significantly higher at baseline. Speculatively, higher anxiety levels at baseline could in turn explain higher MFIS scores at baseline. We also note that sex may impact MFIS scores, since women had higher MFIS scores than men at both baseline and follow-up.

The significance level used in correlation analyses in relation to the number of correlation analyses made in this study could be discussed. Even with our fairly strict significance level Spearman´s rho 0.40 and $p \leq 0.01$ there is a risk of false positive findings. However, the magnitude and the overall pattern of correlation coefficients regarding MFIS scores in relation to other questionnaire data as well as ACTT results are convincing and readers are informed about which correlations that pass Bonferroni correction in the questionnaire and neuropsychological data domains. The strict significance level of 0.01 in most Mann–Whitney *U* tests and the overall pattern of group comparison results in the questionnaire and neuropsychological data domains are also reassuring. As for MFIS scores in relation to biological markers, no false positives can exist since no positive findings at all were detected. MFIS scores in this study are lower than in some previous publications. However, fatigue can hardly be dichotomized as existing or not existing above/below a cut off score on the MFIS scale and our results indicate that MFIS scores in the lower range seem to be clinically relevant, considering that they correlate with other self-reported measures of health as well as with ACTT results. Interestingly, patients fulfilling NEDA-3 at one year follow-up did not show better MFIS scores, or any other self-assessment based scores or neuropsychological test results, than patients not fulfilling NEDA-3. This indicates that the NEDA-3 concept fails to properly reflect important aspects of newly diagnosed CIS and RRMS, like fatigue, mood and cognition, in the short time (one year) follow-up perspective.

In conclusion, fatigue scores in this cohort of patients with newly diagnosed CIS and RRMS were associated with other self-assessment questionnaire data and to some extent with neuropsychological test performances, whereas associations with EDSS, brain volume, number of T2 lesions or protein neurodegenerative/neuroinflammatory markers, including neurofilament light chain levels in CSF, could not be demonstrated. Study results indicate that subjective fatigue scores are not well reflected by some commonly used and objectively measurable disease parameters.

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CRediT authorship contribution statement

Irene Håkansson: Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Lovisa Johansson:** Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Charlotte Dahle:** Data curation, Writing - review & editing. **Magnus Vrethem:** Data curation, Writing - review & editing. **Jan Ernerudh:** Data curation, Writing - review & editing.

Declaration of Competing Interest

Dr. Håkansson, Ms. Johansson and Dr. Ernerudh report no conflict of interest. Dr. Dahle has received honoraria for lectures from Biogen, Teva, Genzyme and Novartis and for advisory boards from Roche, Novartis and Biogen. Dr. Vrethem has received unrestricted research grants from Biogen and Novartis, honoraria for lectures from Biogen and Genzyme and for advisory boards from Roche and Novartis. All authors declare that they have no conflict of interest in relation to the organizations that sponsored this research.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2019.101424.

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