NeuroImage: Clinical 6 (2014) 312–319

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

MRI correlates of disability progression in patients with CIS over 48 months

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ARTICLE INFO

Article history:
Received 17 June 2014
Received in revised form 22 September 2014
Accepted 22 September 2014
Available online 28 September 2014

Keywords:
Multiple sclerosis
Clinically isolated syndrome
MRI
Gray matter
Atrophy
Longitudinal
Lesions

ABSTRACT

Background: Gray matter (GM) and white matter (WM) pathology has an important role in disease progression of multiple sclerosis (MS).
Objectives: To investigate the association between the development of GM and WM pathology and clinical disease progression in patients with clinically isolated syndrome (CIS).
Methods: This prospective, observational, 48-month follow-up study examined 210 CIS patients treated with 30 μg of intramuscular interferon beta-1a once a week. MRI and clinical assessments were performed at baseline, 6, 12, 24, 36 and 48 months. Associations between clinical worsening [24-weeks sustained disability progression (SDP) and occurrence of a second clinical attack] and longitudinal changes in lesion accumulation and brain atrophy progression were investigated by a mixed-effect model analysis after correction for multiple comparisons.
Results: SDP was observed in 32 (15.2%) CIS patients, while 146 (69.5%) were stable and 32 (15.2%) showed sustained disability improvement. 112 CIS patients (53.3%) developed clinically definite MS (CDMS). CIS patients who developed SDP showed increased lateral ventricle volume ($p < .001$), and decreased GM ($p = .011$) and cortical ($p = .001$) volumes compared to patients who remained stable or improved in disability. Converters to CDMS showed an increased rate of accumulation of number of new/enlarging T2 lesions ($p < .001$), decreased whole brain ($p = .007$) and increased lateral ventricle ($p = .025$) volumes.
Conclusions: Development of GM pathology and LVV enlargement are associated with SDP. Conversion to CDMS in patients with CIS over 48 months is dependent on the accumulation of new lesions, LVV enlargement and whole brain atrophy progression.

1. Introduction

Multiple sclerosis (MS) is a chronic, demyelinating, autoimmune disease of the central nervous system (CNS). Although MS was originally considered to be a disease affecting predominantly the white matter (WM), (Geurts et al., 2009; Zivadinov and Pirko, 2012) pathological changes of gray matter (GM) are increasingly recognized as an important determinant of sustained neurological disability and increased relapse activity in MS patients (Fisher et al., 2008; Horakova et al., 2008; Horakova et al., 2012; Zivadinov et al., 2013a; Zivadinov et al., 2013b).

It has been suggested that GM pathology occurs at all stages of the disease, including patients with clinically isolated syndrome (CIS) (Dalton et al., 2004; Calabrese et al., 2007; Ceccarelli et al., 2008; Henry et al., 2008; Audoin et al., 2010; Jure et al., 2010; Raz et al., 2010; Calabrese et al., 2011; Crespy et al., 2011; Roosendaal et al., 2011; Bergsland et al., 2012; Zivadinov et al., 2013b).

Results of previous research in CIS showed a progressive development of global GM, but not WM atrophy, (Dalton et al., 2004; Raz et al., 2010) and a great variability in compartmentalization of GM injury (Jure et al., 2010; Crespy et al., 2011). GM atrophy progression was associated with severity of T2 and T1 lesion burden (Roosendaal et al., 2011). Most importantly, GM atrophy was shown to be an independent predictor of physical disability progression (Calabrese et al., 2011; Crespy et al., 2011; Perez-Miralles et al., 2013) and conversion to clinically definite MS (CDMS) (Calabrese et al., 2011; Zivadinov et al., 2013b) in patients with CIS.

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http://dx.doi.org/10.1016/j.nicl.2014.09.015

Although enormous progress has been made in better understanding of the role of GM pathology in progression of MS patients from the earliest disease stages, there are still some unanswered questions. For example, in spite of the evidence of pre-existing micro- and macroscopic tissue damage already in subjects with radiologically isolated syndrome (RIS) (De Stefano et al., 2011; Giorgio et al., 2011) it is unclear, if there are pre-existing GM pathological processes ongoing in CIS patients before their first clinical onset (Henry et al., 2008; Audoin et al., 2010; Bergsland et al., 2012) or if GM pathology develops predominantly after disease onset (Ceccarelli et al., 2008; Raz et al., 2010).

It is also unclear whether GM pathology develops more rapidly in CIS patients who convert early to CDMS, those that present with dissemination in space and time, or those who develop sustained disability progression (SDP) (Calabrese et al., 2011; Zivadinov et al., 2013b). Most importantly, the majority of the current evidence is based on the cross-sectional (Calabrese et al., 2007; Ceccarelli et al., 2008; Audoin et al., 2010) or longitudinal studies with limited sample size and short follow-up in patients without the use of disease-modifying treatment (DMT) (Dalton et al., 2004; Rocca et al., 2008; Raz et al., 2010). Therefore, the prognostic role of GM pathology for the development of clinical progression from disease onset remains to be elucidated (Calabrese et al., 2011; Roosendaal et al., 2011; Perez-Miralles et al., 2013; Zivadinov et al., 2013b).

An investigator-initiated, prospective, observational study (Bergsland et al., 2012; Kalincik et al., 2012; Horakova et al., 2013; Kalincik et al., 2013; Weinstock-Guttman et al., 2013a; Weinstock-Guttman et al., 2013b; Zivadinov et al., 2013b) of early intramuscular interferon beta-1a treatment in high-risk subjects after CIS (SET study, clin.gov # NCT01592474) was originally conducted to determine clinical, MR imaging, genetic, and environmental outcomes associated with the disability progression and conversion to CDMS over 48 months. Here, we present the extension of previous results after 48 months of follow-up. The objective of this study was to investigate the evolution of GM atrophy and its relationship to clinical disability progression and development of CDMS.

2. Methods

2.1. Study population

The SET study included 220 CIS patients in 8 centers between the years of 2005 and 2009, who were 18–55 years of age, enrolled within 4 months from the clinical event, had an Expanded Disability Status Scale (EDSS) score of 3.5 or less, displayed the presence of two or more T2-hyperintense lesions on diagnostic MRI, and had the presence of two or more oligoclonal bands in cerebrospinal fluid (CSF) obtained at the screening visit prior to steroid treatment (Kalincik et al., 2012; Zivadinov et al., 2013b). The exclusion criteria for this study were lack of clinical and MRI follow-up data after baseline or pregnancy.

The study included clinical visits every 3 months for 48 months and subsequent long-term follow-up in routine clinical practice. Disability was assessed at baseline and every 6 months thereafter, while SDP was determined after 24 weeks from the 48 month examination. MRI examination was obtained at baseline, 6 months, and yearly thereafter. All patients started the treatment at baseline with 30 mg of intramuscular interferon beta-1a once a week, which has been shown to delay conversion to CDMS (Jacobs et al., 2000). All patients were treated with 3–5 g of methylprednisolone for the first symptom before study entry, and a baseline MR examination was performed at least 30 days after steroid administration. Relapses were treated with 3–5 g of methylprednisolone during the study. The treatment changes were made in accordance with the SET study protocol: patients showing inadequate treatment response (i.e. 2 moderate relapses or 6-month sustained progression of one EDSS step during 12 months on treatment) or lack of tolerance (unacceptable flu-like symptoms despite symptomatic treatment or a 3-fold increase in liver enzyme concentrations). The study protocol was approved by the local ethics committees in all participating centers, and all patients gave their informed consent.

2.2. MRI acquisition and analysis

MRI was performed with a standardized protocol on the same 1.5-T scanner (Gyroscan; Philips Medical Systems, Best, the Netherlands). Axial brain acquisitions included fluid attenuated inversion recovery, three-dimensional (3D) T1-weighted images, and T1 spin-echo images before and 5 min after a single injection of 0.1 mmol/kg of gadopentetate dimeglumine. The details of the MRI sequences are provided elsewhere (Zivadinov et al., 2013b).

All MRI scans were interpreted in a blinded manner. Image analyses included a cumulative number and volume of contrast enhanced (CE) and new and enlarged T2 lesions, and analyses of changes in whole-brain and tissue specific global and regional GM volumes (Zivadinov et al., 2013b). All 3D T1 images were preprocessed using an in-house developed lesion inpainting algorithm to minimize the impact of WM lesions on tissue volumetric analyses. Percent changes in whole brain (WB) volumes were obtained using the SIENA method, (Smith et al., 2001) while for the GM, WM, cortical, and lateral ventricle volume changes, we applied a modified SIENAX multi-time point algorithm, as previously described (Dwyer et al., 2014a). Percentage volume changes for the total subcortical deep gray matter (SDGM) (defined as the sum of thalamus, caudate nucleus, putamen, globus pallidus, hippocampus, amygdala, and nucleus accumbens) and thalamus at each time point were estimated using FMRIB's Integrated Registration and Segmentation Tool (FIRST) (Patenaude et al., 2011; Zivadinov et al., 2013b).

All MRI analyses underwent multi-level quality control and were reviewed by a trained operator (NB) at all critical points, and either corrected, if possible, or excluded from further analysis. Brain and skull extraction errors were corrected manually or by adjusting appropriate (Brain Extraction Tool) BET parameters. For all analyses, the same corrected brain extractions were used. In addition, we also marked as failure any longitudinal analysis pairs producing biologically implausible percent changes in GM, WM, or WB. Although the precise value of biological implausibility is not known and may vary by disease, for the work described here we adopted a standardized cutoff of 5% per year (Dwyer et al., 2014b).

2.3. Statistical analysis

All analyses were performed by using statistical software (SPSS 16.0; SPSS, Chicago, IL) and Statistica 10 (Statsoft, Tulsa, OK, USA). Because of non-normality, as assessed by using the Kolmogorov–Smirnov method, T2 and CE lesion volumes and numbers were logarithmically transformed. Demographic, clinical, and MRI characteristics were compared by using the χ^2 test, Student t-test, and Mann–Whitney U test. Patients were divided into two groups based on their progression to the CDMS within the 48 month time period of the study. CDMS was characterized by the development of a second clinical attack. Patients were also divided into three groups based on their disability progression over 48 months of the study. SDP was defined as a one-point increase on EDSS for patients with a baseline EDSS more than 0 or an increase of 1.5 for patients with the baseline EDSS of 0 sustained over at least 24 weeks after the end of the study. EDSS sustained disability improvement (SDI) was defined as at least a one-point decrease in EDSS (Healy et al., 2013). Dissemination in space and time over the 48 months follow-up was evaluated according to the McDonald 2005 (Polman et al., 2005) and 2010 (Polman et al., 2011) criteria.

Separate longitudinal linear or quadratic mixed-effect models with a random intercept for patients, with and without interaction with time, adjusted for age, gender, time from the first event to baseline assessment, and treatment status over the 48 month follow-up, were used to describe temporal associations between MRI measures and the development of CDMS and change in disability status.

In order to minimize potential effect of pseudo-atrophy during the first months of the study treatment, we performed additional confirmatory mixed-effect model analyses that excluded MRI data between baseline and 6 months, and that used only MRI changes related to the previous time-point (6–12, 12–24, 24–36 and 36–48 months).

In spite of assessment of normal distribution of the data and multilevel quality control of all MRI measures, to further minimize potential confounding effect of MRI measures with high rates of biological MRI changes over 48 months, we excluded these data from an additional confirmatory analysis (Supplement Figs. 1 and 2).

A Benjamini–Hochberg (BH) correction with $p < .05$ was used to minimize the false discovery rate.

3. Results

3.1. Baseline demographic, clinical and MRI characteristics

In this 48 month study, 210 of 220 enrolled CIS patients had the available clinical and MRI follow-up data after baseline and were included in the analyses. Table 1 shows baseline demographic, clinical, and MRI characteristics of the CIS patients, separated by conversion status at the 48 month follow-up. CIS patients who converted to CDMS were younger than stable CIS patients ($p < .001$) and had an increased number of CE lesions ($p = .002$), CE lesion volumes (LVs) ($p = .004$) and CE positivity ($p = .009$) (Table 1). No significant MRI brain volume differences were found between the two groups at baseline.

Table 1
Baseline demographic, clinical and MRI characteristics of CIS patients split by conversion status to clinically definite MS at 48 months.

	CDMS (n = 112)	Stable CIS (n = 98)	p-Value
No. of females[a]	79 (71%)	60 (61%)	0.155[b]
Age at onset in years,	27.0 ± 7.2; 26	30.1 ± 8.2; 30	**<0.001**
Time to baseline in days	85.4 ± 25.2; 87	78.0 ± 21.3; 73.5	**0.022**
EDSS at onset[c]	2.4 ± 1.0; 2.0; (0–6.0)	2.3 ± 0.9; 2.0; (0–5.5)	0.354
EDSS at baseline[c]	1.8 ± 0.7; 1.5; (0.0–3.5)	1.6 ± 0.6; 1.5; (0.0–3.5)	0.069
MSFC at baseline	2.5 ± 0.7; 2.5	2.6 ± 0.6; 2.5	0.319
Type of onset (n)[a]			
Optic neuritis	26 (23%)	28 (29%)	0.376[b]
Sensory/motor	43 (38%)	46 (47%)	0.211[b]
Brainstem/cerebellar	17 (15%)	10 (10%)	0.157[b]
Polysymptomatic	21 (19%)	14 (14%)	0.191[b]
No. of CE lesions	1.8 ± 4.1; 0.0	0.3 ± 0.6; 0.0	**0.002**[d]
No. of CE positivity[a]	41 (37%)	16 (19%)	**0.009**[b]
No. of T2 lesions	13.3 ± 9.4; 11.0	10.4 ± 6.6; 9.0	0.082[d]
No. of patients with ≥9 T2 lesions[a]	68 (61%)	52 (53%)	0.370[b]
CE lesion volume	0.2 ± 0.5; 0.0	0.02 ± 0.05; 0.0	**0.004**[d]
T2 lesion volume	6.2 ± 7.0; 3.6	3.8 ± 4.0; 2.5	0.053[d]
Normalized WB volume	1511.6 ± 75.4; 1514.2	1498.8 ± 64.3; 1500.9	0.305
Normalized GM volume	799.7 ± 45.9; 804.4	785.6 ± 46.5; 783.6	0.087
Normalized WM volume	712.0 ± 41.2; 710.3	713.2 ± 35.0; 714.6	0.817
Normalized cortical volume	625.2 ± 37.6; 629.4	614.1 ± 39.2; 611.8	0.080
Normalized lateral ventricle volume	35.4 ± 10.4; 33.0	36.8 ± 11.6; 34.9	0.484
Total normalized SDGM volume	60.6 ± 43.4; 60.9	60.5 ± 39.1; 59.9	0.863
Normalized thalamus volume	20.7 ± 1.7; 20.7	20.3 ± 1.5; 20.3	0.235

Unless otherwise indicated, all data are reported as mean ± standard deviation, median, except for EDSS, where also range is presented.
Differences between the groups were tested by using the Student t-test, χ^2 test and Mann–Whitney rank sum test. Reported p values are adjusted by using Benjamini–Hochberg correction. In bold are presented p values <0.05.
Legend: EDSS = Expanded Disability Status Scale; MSFC = Multiple Sclerosis Functional Composite; No = number; CE = contrast enhancing; WB = whole brain, GM = gray matter; WM = white matter; SDGM = subcortical deep gray matter. Units of volume are in milliliters.
[a] Data in parentheses are percentages.
[b] χ^2 test
[c] Data in parentheses are ranges.
[d] Mann–Whitney rank sum test.

3.2. Follow-up clinical and treatment characteristics

Over the 48 months follow-up, 112 of 210 patients (53.3%) experienced relapses and developed CDMS. The mean time from baseline to first relapse was 12.6 months (median 7.5; range 0–46 months). The mean annual relapse rate was 0.37 ± 0.47 (standard deviation).

Median EDSS score at disease onset was 2.0 (range 0.0–6.0) and 1.5 (range 0–3.5) at baseline in the whole group. At the 48 month follow-up, the median EDSS score was 1.5 (range 0–6.5) in the CDMS group and 1.5 (range 0–4.0) in the stable CIS group. At the 48 month follow-up, 32 (15.2%) CIS patients had SDP, 146 (69.5%) patients were stable and 32 (15.2%) CIS patients had SDI. At 48 months, 138 (65.7%) CIS patients fulfilled the 2005 McDonald criteria and 171 (81.4%) fulfilled the 2010 McDonald criteria for dissemination in space and time. The evolution of MRI lesion and brain volumetric measures in CIS patients split by fulfilling the McDonald 2010 criteria at baseline is shown in Supplement Table 1.

All 112 (53.3%) CDMS converters received steroid treatment for relapses and 139 (66.2%) patients remained on the assigned treatment with 30 mg of intramuscular interferon beta-1a once a week during the 48 month follow-up. Of the 71 CIS patients who changed treatment status, 63 (30%) switched DMT and 8 (3.8%) discontinued DMT. We found significantly higher WB ($p < .001$), WM ($p = .003$), GM ($p < .001$), cortical ($p < .001$), SDGM ($p = .006$) and thalamus ($p = .003$) volume loss over 48 months in CIS patients who changed treatment status. Also, higher lateral ventricle volume (LVV) enlargement ($p < .001$), number of total new T2 lesions ($p < .001$), T2-LV absolute change ($p = 0.01$), CE-LV absolute change ($p < .001$) and EDSS ($p = .002$) were found in these CIS patients. However, no differences in cumulative number of new CE lesions ($p = .058$) have been found between the two groups. A more detailed description of the treatment characteristics is given in Table 2.

3.3. Changes in MRI outcomes according to development of CDMS status over 48 months

A cumulative mean of 11.6 total new T2 lesions developed in the CDMS group versus 3.4 in the stable CIS group ($p < .001$). Additionally, a significantly increased number of new ($p < .001$), and newly enlarging T2 lesions ($p < .001$) occurred in CDMS converters (Table 2). There was also a significantly increased accumulation of T2- and CE-LVs ($p < .038$) and increased CE positivity over the follow-up ($p = .036$) in CDMS converters (Table 2). The mixed-effect model analysis showed a significant association between the development of CDMS and the accumulation of cumulative number of total new T2 lesions ($p < .001$) (Fig. 1), new T2 lesions and newly enlarging T2 lesions (both $p < .001$), but not with absolute change in T2-lesion volume (LV)($p = .292$). CE positivity ($p = .221$) and number of cumulative CE lesions ($p = .159$) over 48 months were not related to the CDMS development in the mixed-effect model analysis. Similar analysis, in which patients with high biological MRI changes over 48 months were excluded, is shown in Supplement Fig. 1.

CDMS converters showed increased WB ($p = .002$), GM ($p = .006$), cortical ($p = .007$), thalamus ($p = .015$) and WM ($p = .017$) volume loss and LVV enlargement ($p = .002$) over the follow-up (Table 2). In the mixed-effect model analysis, a significant association between the development of CDMS and an increased rate of WB volume loss ($p = .007$) and of LVV enlargement ($p = .025$) was detected (Fig. 1). The progression rate of thalamic ($p = .118$, Fig. 1), cortical ($p = .211$) and GM ($p = .207$) volume loss was not significant in the mixed-effect model analysis. Similar analysis, in which patients with high biological MRI changes over 48 months were excluded, is shown in Supplement Fig. 1.

Table 2
Evolution of MRI lesion and brain volumetric measures in CIS patients split by conversion status to clinically definite MS at 48 months.

	CDMS (n = 112)	Stable CIS (n = 98)	p-Value
Patients changed DMT, no. [ae]	58 (52%)	5 (5%)	<**0.001**[b]
EDSS at 4 years [c]	2.0 ± 1.1; 1.5; (0.0–6.5)	1.5 ± 0.7; 1.5; (0.0–4.0)	<**0.001**
MSFC at 4 years	2.5 ± 0.7; 2.3	2.4 ± 0.6; 2.3	0.379
Cumulative no. of			
Total new T2 lesions	11.6 ± 18.6; 5.5	3.4 ± 4.8; 2.0	<**0.001**[d]
New T2 lesions	8.63 ± 13.6; 4.0	2.7 ± 4.3; 1.0	<**0.001**[d]
Newly enlarging T2 lesions	3.0 ± 5.8; 1.0	0.7 ± 1.3; 0.0	<**0.001**[d]
Cumulative no. of new CE lesions	2.4 ± 8.5; 0.0	0.7 ± 2.2; 0.0	0.065[d]
CE positivity, no. [a]	46 (42%)	29 (30%)	**0.036**[b]
T2 lesion volume absolute change (ml)	0.4 ± 4.7; 0.03	−0.3 ± 1.4; −0.3	**0.038**[d]
CE lesion volume absolute change (ml)	−0.1 ± 0.4; 0.0	−0.004 ± 0.09; 0.0	**0.009**[d]
WB volume % change	−3.0 ± 2.4; −2.7	−2.0 ± 1.6; −1.7	**0.002**
GM volume % change	−2.8 ± 2.5; −2.6	−1.8 ± 1.8; −1.7	**0.006**
WM volume % change	−2.1 ± 2.4; −1.7	−1.3 ± 1.8; −1.3	**0.017**
Cortical volume % change	−3.2 ± 2.5; −2.9	−2.3 ± 1.7; −2.2	**0.007**
Lateral ventricle volume % change	20.3 ± 16.6; 16.6	13.2 ± 9.7; 13.2	**0.002**
Total normalized SDGM volume % change	−4.1 ± 4.0; −3.3	−3.1 ± 2.9; −2.7	0.068
Thalamus volume % change	−5.4 ± 4.5; −4.9	−3.8 ± 3.8; −2.8	**0.015**

Unless otherwise indicated, all data are reported as mean ± standard deviation, median, except for EDSS, where also range is presented. Differences between the groups were tested by using the Student t-test, χ^2 test and Mann–Whitney rank sum test. Reported p values are adjusted by using Benjamini–Hochberg correction. In bold are presented p values <0.05.
Legend: EDSS = Expanded Disability Status Scale; DMT = disease-modifying treatment; MSFC = Multiple Sclerosis Functional Composite; No = number; CE = contrast enhancing; WB = whole brain, GM = gray matter; WM = white matter; LV = lateral ventricle; SDGM = subcortical deep gray matter; ml = milliliters.

[a] Data in parentheses are percentages.
[b] χ^2 test
[c] Data in parentheses are ranges.
[d] Mann–Whitney rank sum test.
[e] Of 71 patients who changed treatment status over the follow-up, 26 patients switched to a high-dose (44 μg) interferon beta-1a, 18 patients switched to natalizumab, 7 patients switched to glatiramer acetate, 4 patients switched to intravenous immunoglobulin E, 3 patients switched to fingolimod, 2 patients switched to subcutaneous interferon beta-1b, 2 patients switched to alemtuzumab, one patient switched to low-dose (22 μg) interferon beta-1a, and 8 patients discontinued DMT.

The mixed-effect model analysis that excluded MRI data between baseline and 6 months showed a significant association between the development of CDMS and the accumulation of cumulative number of total new T2 lesions ($p < .001$), increase of WM ($p = .003$) and GM ($p = .024$) volume loss and LVV enlargement ($p < .001$) over the follow-up. Increased WB volume loss in CDMS patients was not significant after BH correction for multiple comparisons ($p = .036$ before BH correction).

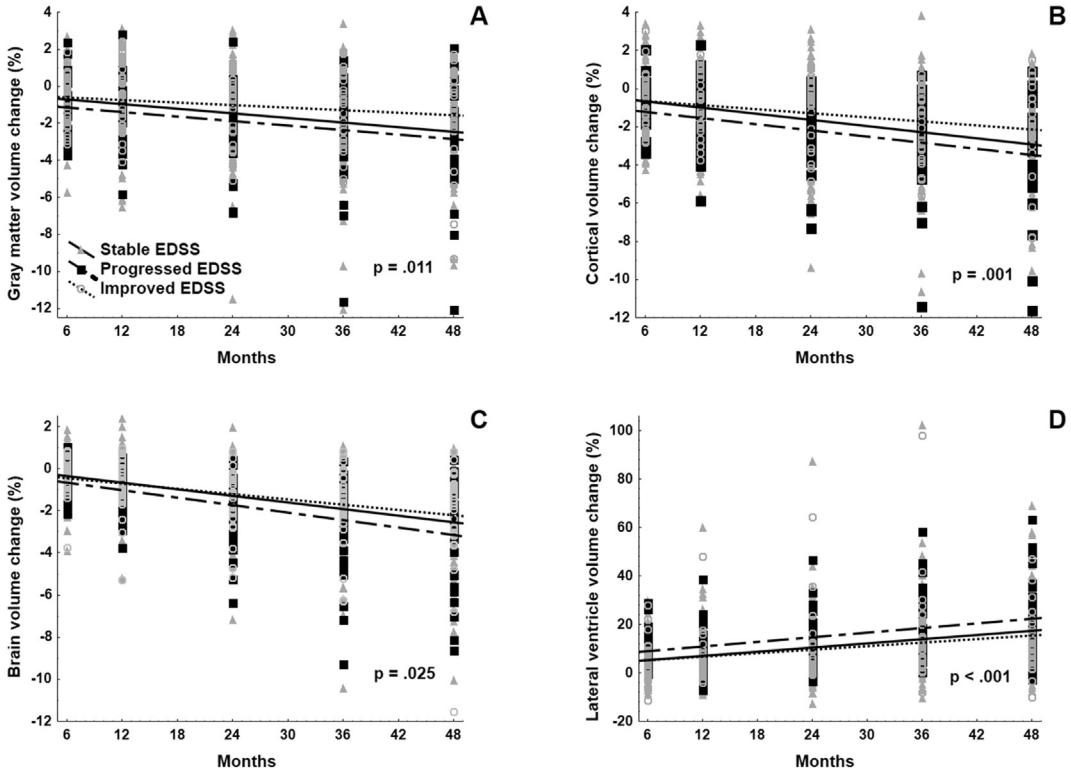

Fig. 1. Changes in lesion activity and lesion volume MRI measures by conversion status to clinically definite multiple sclerosis over time. p values were adjusted by using Benjamini–Hochberg correction to minimize for false discovery rate. A, Percentage change in whole-brain volume ($p = .007$). B, Cumulative number of total new and newly enlarging T2 lesions ($p < .001$). C, Percentage change in thalamic volume ($p = .118$). D, Percentage change in lateral ventricle volume ($p = .025$).

In further confirmatory analysis that considered only MRI changes related to the previous time-point and excluded MRI data between 0 and 6 months, we found a significant association between the development of CDMS and the accumulation of cumulative number of total new T2 lesions ($p < .001$), increased WB volume loss ($p = .033$) and LVV enlargement ($p = .025$).

3.4. Changes in MRI outcomes according to disability status over 48 months

Table 3 and Fig. 2 show the evolution of MRI lesion and brain volumetric measures in CIS patients split by disability status at 48 months.

In the linear mixed-effect model analysis, significantly increased LVV enlargement ($p < .001$), and GM ($p = .011$) and cortical ($p = .001$) volume loss were detected in CIS patients with SDP compared to patients who remained stable or improved in their disability status over 48 months. Similar analysis, in which patients with high biological MRI changes over 48 months were excluded, is shown in Supplement Fig. 2.

The mixed-effect model analysis that excluded MRI data between baseline and 6 months showed significantly increased LVV enlargement ($p = .002$), and cortical ($p = .002$), WB ($p = .014$) and GM ($p = .022$) volume loss in CIS patients with SDP compared to patients who remained stable or improved in their disability status over 48 months.

In further confirmatory analysis considering MRI changes related to the previous time-point, that excluded MRI data between 0 and 6 months, we did not find any significant relationships between MRI outcomes and disability status.

4. Discussion

This study presents a prospective, longitudinal investigation of the association between the progression of GM atrophy and the accumulation of disability and conversion to CDMS in patients receiving weekly interferon beta-1a treatment. To the best of our knowledge, this is the first follow-up study reported to date on the evolution of GM pathology and development of SDP in a homogeneous sample of CIS patients treated with DMT. Although previous studies have reported a relationship between physical disability and development of GM pathology, (Dalton et al., 2004; Calabrese et al., 2007; Henry et al., 2008; Calabrese et al., 2009; Audoin et al., 2010; Raz et al., 2010) they did not use SDP assessment, were of limited sample size and employed a shorter follow-up period.

The most important finding of this study suggests that SDP is associated with the development of GM atrophy in CIS patients. More specifically, we have found significantly increased GM, and cortical atrophy in patients with SDP compared to patients who remained stable or improved in their disability status over 48 months. This confirms the results of previous cross-sectional (Calabrese et al., 2007; Henry et al., 2008; Audoin et al., 2010; Jure et al., 2010) and longitudinal CIS studies, (Dalton et al., 2004; Rocca et al., 2008; Raz et al., 2010) as well as longitudinal studies performed in early relapsing–remitting MS cohorts (Horakova et al., 2008; Calabrese et al., 2012; Zivadinov et al., 2013a; Jacobsen et al., 2014). Our results were confirmed by an additional analysis that excluded MRI measures over the first 6 months of the study to minimize the potential effect of pseudo-atrophy. However, further investigation is needed to explore whether accelerated GM volume loss in SDP patients occurring early after disease onset can be attributed to early neurodegenerative processes or rather to greater pseudo-atrophy reflecting higher inflammatory activity at disease onset. Given that pseudo-atrophy occurring after DMT initialization appears to be driven more by WM volume loss (Vidal-Jordana et al., 2013) this question remains to be elucidated.

Based on our previous research showing a relationship between thalamic atrophy and conversion to CDMS, (Zivadinov et al., 2013a) the finding of relatively high thalamic and SDGM volume loss also in the SDI group is unexpected. Therefore further investigation is needed to clarify if disability progression in CIS patients depends more on cortical rather than deep GM pathology or whether these findings were influenced by the low number of SDI patients. Interestingly, patients with SDI showed the lowest cortical, GM and WB atrophy development, which is in line with findings of milder cortical lesion burden accumulation in patients with a benign MS course (Calabrese et al., 2009; Calabrese et al., 2013). Increased LVV enlargement together with an increased rate of brain volume loss, that was driven mostly by global GM rather than by WM pathology in patients with SDP, was also recently reported in MS patients over 10 years (Jacobsen et al., 2014). Most importantly, neither accumulation of cumulative T2 or CE lesions or their LVs were associated with development of SDP, which further emphasizes that GM pathology plays a central role in irreversible disability progression of MS patients.

Another interesting result of this study indicates an increased rate of WB atrophy progression and accumulation of T2 lesions in CIS patients who converted to CDMS. In this extension of the original SET study, the

Table 3
Evolution of MRI lesion and brain volumetric measures in CIS patients split by sustained disability progression status at 48 months.

	SDP (n = 32)	Stable (n = 146)	SDI (n = 32)
Cumulative no. of			
Total new T2 lesions	10.9 ± 24.0; 3.0	6.6 ± 9.7; 3.0	9.7 ± 18.5; 3.0
New T2 lesions	7.4 ± 16.2; 2.0	5.0 ± 8.3; 2.0	7.6 ± 13.2; 2.0
New enlarging T2 lesions	3.5 ± 8.3; 1.0	1.5 ± 2.4; 0.0	2.0 ± 5.9; 0.0
Cumulative no. of new CE lesions	4.1 ± 14.6; 0.0	1.0 ± 2.3; 0.0	1.2 ± 3.0; 0.0
CE positivity, no. [a]	8 (24%)	55 (38%)	12 (38%)
T2 lesion volume absolute change (ml)	0.9 ± 6.7; −0.04	−0.3 ± 2.5; −0.3	0.9 ± 2.8; 0.08
CE lesion volume absolute change (ml)	−0.09 ± 0.2; 0.0	−0.08 ± 0.3; 0.0	−0.01 ± 0.2 0.0
WB volume % change	**−3.0 ± 2.4; −2.4** [b]	−2.5 ± 1.9; −2.1	−2.1 ± 2.3; −1.5
GM volume % change	**−2.9 ± 2.9; −2.7** [b]	−2.3 ± 2.0; −2.0	−1.6 ± 2.5; −1.4
WM volume % change	−2.1 ± 2.5; −1.9	−1.6 ± 2.0; −1.3	−1.8 ± 2.4; −1.2
Cortical volume % change	**−3.5 ± 2.9; −3.0** [b]	−2.8 ± 2.0; −2.6	−2.1 ± 2.3; −2.1
Lateral ventricle % change	**22.0 ± 17.0; 18.7** [b]	16.5 ± 13.6; 14.3	13.8 ± 12.6; 11.0
Total normalized SDGM volume % change	**−3.4 ± 4.7; −2.8**	−3.5 ± 3.3; −3.0	−4.5 ± 3.5; −3.9
Thalamus volume % change	**−4.8 ± 4.9; −4.1**	−4.4 ± 4.0; −4.0	−5.2 ± 4.8; −4.6

Unless otherwise indicated, all data are reported as mean ± standard deviation, median. Differences between the SDP, stable and SDI groups were tested by using the mixed-effect model analysis. Reported p values are adjusted by using Benjamini–Hochberg correction. In bold are presented p values <0.05 for differences between SDP and stable plus SDI group.
Legend: SDP = sustained disability progression; SDI = sustained disability improvement; No = number; CE = contrast enhancing; WB = whole brain, GM = gray matter; WM = white matter; SDGM = subcortical deep gray matter; ml = milliliters.
[a] Data in parentheses are percentages.
[b] p values < 0.05 for differences between SDP and stable plus SDI group in the confirmatory analysis that excluded MRI data between baseline and 6 months.

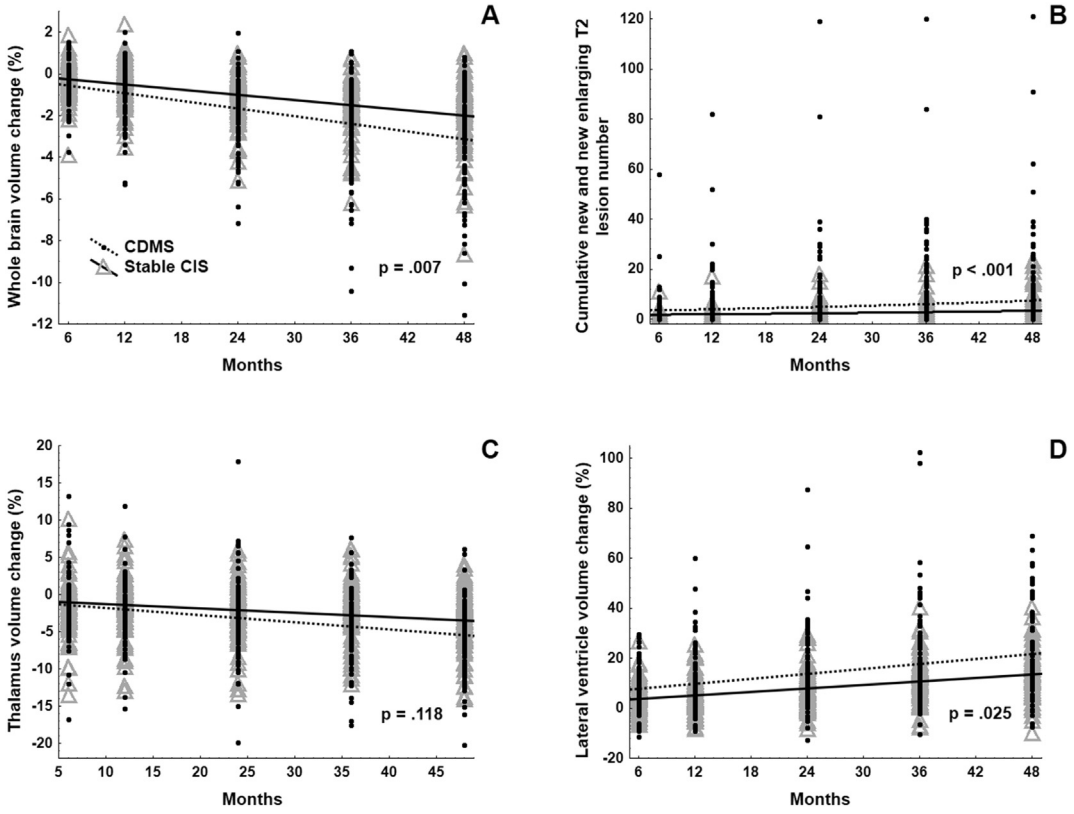

Fig. 2. Temporal changes in global and tissue-specific MRI measures by disability progression status at different time points of the study are shown as a mean ± standard error. p values were adjusted by using Benjamini–Hochberg correction to minimize for false discovery rate. A, Percentage change in gray matter volume ($p = .011$). B, Percentage change in cortical volume ($p = .001$). C, Percentage change in whole brain volume ($p = .025$). D, Percentage change in lateral ventricle volume ($p < .001$).

conversion to CDMS was not found to be associated with thalamus pathology, a finding previously reported over the 2-year follow-up (Zivadinov et al., 2013b). We hypothesize that thalamus pathology is presumably a very early marker of brain damage whose predictive value dissolves along with disease progression and brain tissue damage accumulation, (Minagar et al., 2013) as also reported in two recent studies (Zivadinov et al., 2013a; Jacobsen et al., 2014). Furthemore, LVV enlargement was more advanced in CIS patients who developed SDP, as well as in those who converted to CDMS. Further investigation is needed to evaluate the pathogenesis of LVV enlargement in CIS patients, which may be also related to altered CSF flow pulsatility (Magnano et al., 2012).

Although the use of DMT has been shown to delay disability progression and conversion to CDMS, conventional DMT has an unsatisfactory efficacy to suppress disease activity in specific subpopulations of MS patients (Havrdova et al., 2010). All CIS patients enrolled in this study were treated with intramuscular interferon beta-1a and almost 70% of patients remained on the assigned treatment during the 48 month follow-up. While the use of DMT probably reduced disease progression, it failed to prevent GM atrophy development in a considerable proportion of study subjects. The results of the present study showed that CIS patients who changed their treatment status over 48 months developed more advanced GM and WM pathology of the brain. However, it is important to note that this finding might be due not only to several factors, such as a more aggressive course of the disease associated with more pronounced inflammation but also to a more significant pseudoatrophy effect caused by switching of different DMTs used in patients with more aggressive disease.

In this context, it has been suggested that different mechanisms leading to cortical demyelination and neurodegeneration occur independently of WM pathology (Geurts et al., 2009; Popescu and Lucchinetti, 2012; Zivadinov and Pirko, 2012). Pathological studies have shown that GM pathology in early MS can be attributed to both active inflammatory cortical demyelination and neuronal loss following retrograde degeneration from WM (Geurts et al., 2009; Popescu and Lucchinetti, 2012; Zivadinov and Pirko, 2012). It was shown that meningeal inflammation also contributes to cortical demyelination in early MS (Popescu and Lucchinetti, 2012). In contrast to chronic progressive stages of MS, early MS is characterized by highly inflammatory, but usually promptly resolving cortical lesions (Popescu and Lucchinetti, 2012). However, cortical lesions in early MS may also be associated with cortical oligodendrocyte degeneration, demyelination and neuronal damage (Popescu and Lucchinetti, 2012; Zivadinov and Pirko, 2012). While cortical demyelination and neurodegeneration in the progressive stages of the disease are mainly driven by oxidative injury, it is not fully understood whether these mechanisms are also relevant to the early stages of the disease (Fischer et al., 2013). However, it has been shown that excessive oxidative injury may be related to the MS-specific gene expression changes of molecular pathways associated with inflammation and oxidative stress that result in DNA damage and alterations of regenerative mechanisms affecting glial and neuronal cell processes in the cerebral cortex of MS patients (Fischer et al., 2013). The present study was limited to identifying pathophysiological mechanisms determining brain tissue damage. Only WM focal damage and global, tissue specific and regional atrophy were assessed, while more sophisticated MRI techniques like diffusion-tensor imaging, magnetization transfer imaging or magnetic resonance spectroscopy were not applied. Hence, further research is needed to clarify the nature and extent of GM pathology in CIS and to investigate the effect of newly introduced DMTs on the prevention of GM pathology. In particular, future investigations of DMT should focus on the prevention of cortical and SDGM pathology in the early stages of MS.

The strengths of this study include a large sample size, homogeneous treatment, relatively long follow-up, frequent serial MRI assessment on a scanner that did not undergo any changes over the follow-up and

assessment of SDP after 24 weeks, following the 48 month follow-up. As compared to linear or logistic regression, the application of mixed-effect models analysis with and without interaction with time, adjusted for age, gender, time from the first event to baseline assessment, and treatment status over the 48 month follow-up, increased the statistical power of the study.

However, there are also limitations of the present study. The absence of healthy controls prevented us from exploring GM atrophy progression related to normal aging. In addition, given that all CIS patients entering the SET study had two or more oligoclonal bands in their CSF and two or more hyperintense T2 lesions at disease onset, they represent a high risk population for disease progression, which limits the generalizability of our findings to all CIS patients. Regional approaches, including voxel-based morphometry and T2 lesion distribution probability may provide additional information in explaining the conversion to CDMS and disability progression. In addition, it is well known that high-dose intravenous corticosteroid (Fox et al., 2005), interferon-beta (Hardmeier et al., 2005) or natalizumab (Vidal-Jordana et al., 2013) treatment leads to a temporary reduction in brain volume, mainly due to WM volume loss, (Vidal-Jordana et al., 2013) the phenomenon described as a pseudoatrophy (Zivadinov et al., 2008). The pseudoatrophy effect is present in the first 3 months of the study when interferon-beta treatment is used (Dwyer et al., 2014b). It is therefore less likely that pseudoatrophy would have a significant effect on brain volume changes over 48 months in the present study. Moreover, all CIS patients were treated with weekly intramuscular interferon beta-1a since baseline and analyses were adjusted for treatment change. In addition, MRI scans were performed at least 30 days after high-dose intravenous corticosteroid administrations.

In conclusion, this study showed that the development of cortical GM atrophy is associated with the development of SDP in CIS patients on a standard DMT. This study extends and strengthens previous evidence of a strong relationship between GM pathology and disability progression in CIS.

Supplementary data related to this article can be found online at http://doi.dx.org/10.1016/j.nicl.2014.09.015.

Disclosure

The SET study was supported by the Czech Ministries of Education and Health (NT13237-4/2012, PRVOUK-P26/LF1/4, RVO-VFN64165/2012) and Biogen Idec.

Conflicts of interest

Mr. Bergsland and Dr. Ramasamy report no disclosures. Dr. Uher received financial support for conference travel and honoraria from Biogen Idec. Dr. Horakova received compensation for travel, speaker honoraria and consultant fees from Biogen Idec, Novartis, Merck Serono, Bayer Schering, and Teva, as well as support for research activities from Biogen Idec. Dr. Tyblova received compensation for travel and honoraria from Biogen Idec, Sanofi Aventis, Teva and Merck Serono. Drs. Seidl, Vaneckova, and Krasensky received financial support for research activities from Biogen Idec. Dr. Havrdova received speaker honoraria and consultant fees from Biogen Idec, Merck Serono, Novartis, Genzyme and Teva, as well as support for research activities from Biogen Idec and Merck Serono. Dr. Zivadinov received financial support for research activities from Teva Pharmaceuticals, Biogen Idec, Claret Medical, Genzyme, Novartis and Greatbatch. He received personal compensation from, Biogen Idec, Novartis, Genzyme and EMD Serono for speaking and consultant services.

Acknowledgments

The authors thank the patients who participated in this study. We thank the other Clinical Centers and investigators who participated in the SET study: i) M. Vachova, S. Machalicka, and J. Kotalova from KZ a.s. Hospital, Teplice; ii) Y. Benesova, P. Praksova, and P. Stourac from University Hospital, Brno, Bohunice; iii) M. Dufek from St. Anne's University Hospital, Brno; iv) E. Meluzinova, J. Pikova, and E. Houzvickova from Charles University in Prague, 2nd Faculty of Medicine, Motol; v) D. Zimova from Charles University in Prague, 3rd Faculty of Medicine, Kralovske Vinohrady; vi) J. Sucha from University Hospital, Plzeň; and vii) V. Sladkova and J. Mares from University Hospital, Olomouc.

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