SPINAL CORD LESIONS AND BRAIN GREY MATTER ATROPHY INDEPENDENTLY PREDICT CLINICAL WORSENING IN DEFINITE MULTIPLE SCLEROSIS: A 5-YEAR, MULTICENTRE STUDY

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ABSTRACT

Objectives. To evaluate the combined contribution of brain and cervical cord damage in predicting 5-year clinical worsening in a multicentre cohort of definite multiple sclerosis (MS) patients.

Methods. Baseline 3.0 T brain and cervical cord T2- and 3D T1-weighted MRI was acquired in 367 MS patients (326 relapse-onset, 41 progressive-onset) and 179 healthy controls. Expanded Disability Status Scale (EDSS) score was obtained at baseline and after a median follow-up of 5.1 years (interquartile range=4.8-5.2). At follow-up, patients were classified as clinically stable/worsened according to EDSS changes. Generalized linear mixed models identified predictors of clinical worsening, evolution to secondary progressive (SP) MS, and reaching EDSS=3.0, 4.0 and 6.0 milestones at 5 years.

Results. At follow-up, 120/367 (33%) MS patients worsened clinically; 36/256 (14%) relapsing-remitting MS patients evolved to SPMS. Baseline predictors of EDSS worsening were progressive- *vs* relapse-onset MS (standardized beta [β]=0.97), higher EDSS (β=0.41), higher cord lesion number (β=0.41), lower normalized cortical volume (β=-0.15) and lower cord area (β=-0.28) (C-index=0.81). Older age (β=0.86), higher EDSS (β=1.40) and cord lesion number (β=0.87) independently predicted SPMS conversion (C-index=0.91). Predictors of reaching EDSS=3.0 after 5-years were higher baseline EDSS (β=1.49), cord lesion number (β=1.02) and lower normalized cortical volume (β=-0.56) (C-index=0.88). Baseline age (β=0.30), higher EDSS (β=2.03), higher cord lesion number (β=0.66), and lower cord area (β=-0.41) predicted EDSS=4.0 (C-index=0.92). Finally, higher baseline EDSS (β=1.87) and cord lesion number (β=0.54) predicted EDSS=6.0 (C-index=0.91).

Conclusions. Spinal cord damage and, to a lesser extent, cortical volume loss helped predicting worse 5-year clinical outcomes in MS.

KEY MESSAGES

What is already known on this topic:

- Multiple sclerosis (MS) is characterized by heterogeneous clinical manifestations, imaging features, and disease evolution. Thanks to their sensitivity to demyelination and neurodegeneration, MRI-derived measures are good candidates for the identification of the mechanisms associated with disease progression in MS patients.
- Only a limited number of MRI studies has combined the analysis of measures of brain and spinal cord damage to identify the factors associated with MS worsening.

What this study adds:

- In this study, we evaluated the combined contribution of brain and cervical cord MRI damage in predicting 5-year clinical worsening in a multicentre cohort of 367 definite MS patients.
- A multivariate model including spinal cord lesions, spinal cord atrophy and cortical atrophy was able to identify patients showing clinical disability progression at 5 years
- Cord lesion number was relevant also to explain evolution to secondary progressive MS and reaching different clinical disability milestones.

How this study might affect research, practice or policy:

- The combined assessment of brain and spinal cord damage may better identify MS patients who will have medium-term disease progression, supporting a better treatment decision and optimizing patients' management.

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory, demyelinating and neurodegenerative CNS disease with heterogeneous evolution and clinical/imaging features.[1] Although MS pathological hallmark is the accumulation of focal demyelinating lesions, brain and spinal cord neurodegeneration also occurs.[1]

Given the expanding therapeutic scenario of MS, a more precise identification of patients with unfavorable clinical outcome would optimize patients' management. Thanks to their sensitivity to focal lesions (i.e., demyelination) and atrophy (i.e., neurodegeneration), MRI-derived measures are good candidates for identifying mechanisms associated with disease progression.

Longitudinal work has shown that, in patients with clinically isolated syndromes, the presence, number and topography of brain and spinal cord T2-hyperintense lesions relates to the conversion to clinically definite MS and disability accumulation over the long-term.[2-4] In definite MS, T2-weighted[5-8] and T1-weighted[6, 9, 10] MRI brain abnormalities have been associated with more severe disability at medium- and long-term follow-up.[5-10] Conversely, only a few studies found that spinal cord lesions predicted long-term disability.[5, 9, 11]

Brain atrophy is well-known in MS.[12] Grey matter (GM) loss is present at all stages but is more severe in progressive forms, contributing to explain clinical disability.[12] Available data indicate that whole-brain or GM volume loss is more relevant than other MRI measures (e.g., focal lesions or white matter [WM] volume loss) to predict subsequent disability accrual.[10, 13-21] Importantly, this holds true not only for whole-brain or GM volumetry,[10, 14, 15, 18, 20] but also for strategic GM structures, such as deep GM[13, 16, 21] or relevant cortical regions/networks.[17, 20]

Spinal cord tissue loss, especially in the cervical segment, occurs to a great extent in MS and is associated with concurrent clinical disability.[22] While a significant relationship is present between cord tissue loss and accumulating disability in the short-term,[21, 23, 24] this association disappears at

longer clinical follow-up (more than 10 years), suggesting that spinal cord tissue loss may be critical for clinical deterioration in the first years of the disease.[10, 15, 25]

Only a few MRI studies combined brain and spinal cord damage to identify the factors associated with MS worsening, providing inconclusive results, mainly because only a subset of variables (i.e., lesions or atrophy) was analyzed. Indeed, worsening of clinical disability over time has been associated to focal lesions in the brain[5, 8, 9] and cervical cord,[5, 9] as well as to brain[10, 18] and cord tissue loss.[10, 18] In this perspective, the multiparametric evaluation of both focal demyelinating lesions and irreversible tissue loss is likely to clarify the relative contribution of different pathological processes to disability progression.

To address this issue, we prospectively collected five year follow-up clinical data from a multicenter cohort of definite MS patients, who participated in a previous MRI study characterizing cervical cord atrophy at different stages of the disease.[26] In that study, baseline measures of brain focal lesions and atrophy were also computed.[26] The main goal of the present investigation was therefore to evaluate the independent role of brain and cervical cord damage in predicting medium-term clinical outcomes in this cohort.

METHODS

Ethics committee approval. Approval was received from the local ethical committee (IRCCS San Raffaele Scientific Institute, Milan, Italy; protocol ID: 24/INT/2015). All subjects gave written informed consent prior to study participation. A MAGNIMS data-sharing agreement was signed among the participating centres.

Study population. Participants are part (84%) of a prospective cohort, enrolled between May 2010 and March 2016 at 9 MAGNIMS European sites (www.magnims.eu).[26] Detailed inclusion/exclusion criteria are reported elsewhere.[26] At the time of inclusion, patients had to have

stable disease-modifying treatment during the last 6 months and received no corticosteroids during the last month. Patients were contacted to undergo a medium-term follow-up clinical evaluation after at least 3 years from baseline clinical and MRI assessment. The final cohort clinically reassessed at follow-up included 367 MS patients (148 males/219 females; mean age=46.0 years, interquartile range [IQR]=37-55 years). Of these, 326 were relapse-onset MS (256 relapsing-remitting and 70 secondary progressive [SP] MS) and 41 were progressive-onset MS. Baseline MRI data from 179 healthy controls (HC) (75 males/104 females; mean age=39.6 years; IQR=26-52 years) were also included.

Clinical assessment. MS patients underwent a complete neurological evaluation at baseline, in which the Expanded Disability Status Scale (EDSS) score[27] was rated and disease-modifying treatment (DMT) status was recorded. The follow-up neurological assessment was performed after a median of 5.1 years (IQR=4.8-5.2 years) and included rating of EDSS score, occurrence of clinical relapses and changes of DMT (binary coded). At follow-up, patients were clinically worsened if they had an EDSS score increase ≥1.5 points when baseline EDSS was 0, ≥1.0 point when baseline EDSS was between 1.0 and 5.5 or ≥0.5 points when baseline EDSS was ≥6.0.[17] According to routine clinical practice, EDSS changes were confirmed during a second clinical assessment, performed after a 3-month, relapse-free period. In relapse-onset MS, conversion to SPMS was defined[9, 17] as the development of irreversible EDSS increase, retrospectively observed over a period of at least 6 months, independent from relapses.

MRI acquisition and analysis. At baseline, all participating centres used a 3.0 T scanner (Hospital Vall d'Hebron, Barcelona: Siemens Magnetom Trio; UCL London and IRCCS San Raffaele Scientific Institute: Philips Achieva; Neurocenter of Southern Switzerland, Lugano, and University of Heidelberg, Mannheim: Siemens Magnetom Skyra; University of Campania "L. Vanvitelli", Naples: General Electric Signa HDtx; University of Oxford: Siemens Magnetom Prisma) to acquire

the following MRI sequences:[26] 1) Brain: a) dual-echo (DE) fast spin-echo or 2D/3D T2-weighted fluid-attenuated inversion recovery; and b) sagittal three-dimensional (3D) T1-weighted scan; 2) Cervical cord: c) short-tau inversion recovery (STIR) or T2-weighted/DE fast spin-echo; and d) sagittal 3D T1-weighted scan. Inversion preparation was applied to cord and brain 3D T1-weighted scans to enhance WM/GM contrast; spatial resolution of these sequences was about 1 mm^3 at all sites.

Brain T2-hyperintense and T1-hypointense lesion volumes (LV) were calculated using a semi-automated method included in Jim 7.0 (Xinapse Systems, Colchester, UK), as previously reported.[26] Normalized brain (NBV), cortical GM (NcGMV), deep GM (NDGMV) and WM volumes (NWMV) were calculated on lesion-filled 3D T1-weighted images using FSL SIENAx and FIRST. The number of cervical cord lesions was counted on STIR/DE scans by one experienced observer, and normalized cord cross-sectional area (CSAn) between C1/C2 and C7 was calculated on cord 3D T1-weighted images using the active surface method included in Jim 7.0, and by adjusting cord area to the baseline FSL SIENAx brain scaling factor.[26]

Statistical analysis. T2 and T1 LV were log transformed. Comparisons of baseline demographic, clinical and MRI measures between HC and MS patients, as well as among HC, relapse-onset and progressive-onset MS, were performed using the Fisher's exact and Mann-Whitney, linear models and age- and sex-adjusted generalized linear mixed-effects models accounting for site heterogeneity and clustering (subjects within sites), using random intercepts.

Generalized linear mixed-effects models, including follow-up duration, baseline DMT, and DMT change during the follow-up as covariates and random intercepts for site, were run to investigate the potential role of each demographic, clinical and brain/cord MRI variable as univariate predictors of clinical disability worsening in all MS patients, conversion to SPMS in relapse-onset MS patients, and reaching of the following milestones of the EDSS score, which have been considered as major benchmarks of disease evolution:[28] a) EDSS=3.0 (fully ambulatory, with

mild disability in 3 or 4 functional systems [FS] or moderate disability in one FS); b) EDSS=4.0 (ambulatory for at least 500 meters, despite severe disability in one FS, or other combinations of moderate disability in other FS); and c) EDSS=6.0 (unilateral assistance to walk 100 meters). This latter analysis was run only on the subsets of MS patients having a baseline EDSS score below the given milestones. Standardized beta and associated ORs were reported. Since univariate analysis was intended for explorative purpose, no correction for multiplicity was carried out, given also the limited number of examined predictors.

Then, generalized linear mixed models with L1-penalized variable selection[29] identified the variables independently predicting 5-year disability worsening, SPMS conversion, and reaching EDSS=3.0, 4.0 and 6.0 milestones, among all demographic, clinical and brain/cord MRI study variables. The optimal value of the tuning parameter, which controls the amount of penalty and promotes variable selection, was chosen according to Akaike Information Criterion (AIC). We included follow-up duration, baseline DMT and DMT change during the follow-up as confounding covariates and random intercepts for site. Standardized beta coefficients from the final models including non-zero parameters are reported. Model discrimination, i.e., the ability to distinguish subjects undergoing an event from those who are not, was assessed by computing the AUC (C-index).

All statistical analyses were performed using SAS release 9.4 and R version 4.1.1.

RESULTS

Demographic, clinical and MRI assessment. Table 1 summarizes baseline demographic, clinical and MRI variables of the subjects included in the study.

Table 1. Main demographic, clinical and MRI measures of healthy controls (HC) and patients with multiple sclerosis (MS), first considered as a whole and then divided according to disease onset.

	HC (n=179)	**MS patients (n=367)**	**p***	**Relapse-onset MS patients (n=326)**	**Progressive-onset MS patients (n=41)**	**p** (p***)**
Men (%) Women (%)	75 (42) 104 (58)	148 (40) 219 (60)	0.78[a]	121 (37) 205 (63)	27 (66) 14 (34)	<0.001[a] (0.002[a])
Mean age (IQR) [years]	39.6 (26-52)	46.0 (37-55)	<0.001[c]	44.8 (36-54)	55.0 (47-63)	<0.001[c] (<0.001[c])
Median disease duration (IQR) [years]	-	13.0 (5.1-20)	-	13.0 (4.6-20)	13.0 (7.7-20)	- (0.34[b])
Median EDSS (IQR)	-	3.0 (1.5-5.0)	-	3.0 (1.5-4.5)	6.0 (5.0-6.5)	- (<0.001[b])
Baseline DMT^: -none (n) -1st line (n) -2nd line (n)	-	 140 143 84	-	 108 142 76	 32 1 8	- (<0.001[a])
Median follow-up duration (IQR) [years]	-	5.1 (4.8-5.2)	-	5.1 (4.8-5.2)	5.2 (4.9-5.1)	- (0.6[b])
Mean ARR at follow-up (SD)	-	0.06 (0.16)	-	0.07 (0.17)	0.01 (0.05)	- (<0.001[c])
DMT change: -yes (%) -no (%)	-	 58 (16) 309 (84)	-	 56 (17) 270 (83)	 2 (5) 39 (95)	- (<0.041[a])
Mean T2 LV (SD) [ml]	0.02 (0.9)	9.6 (11.2)	<0.001[d]	9.6 (11.2)	9.7 (11.2)	<0.001[d] (0.22[d])
Mean T1 LV (SD) [ml]	0.00 (0.1)	6.5 (8.1)	-	6.4 (8.0)	7.1 (8.7)	<0.001[d] (0.25[d])
Mean NBV (SD) [ml]	1475 (65)	1421 (79)	<0.001[d]	1425 (81)	1395 (64)	<0.001[d] (0.39[d])
Mean NcGMV (SD) [ml]	615 (47)	594 (42)	<0.001[d]	597 (42)	570 (31)	<0.001[d] (0.48[d])
Mean NWMV (SD) [ml]	687 (39)	670 (48)	<0.001[d]	669 (49)	673 (42)	<0.001[d] (0.15[d])
Mean NDGMV (SD) [ml]	54 (4)	49 (7)	<0.001[d]	49 (7)	47 (5)	<0.001[d] (0.12[d])
Median cervical cord lesion number (IQR)	-	2 (1-4)	-	2 (1-4)	3 (2-6)	- (0.04[e])
Mean cord CSAn (SD) [mm^2]	76.6 (6.7)	70.9 (8.8)	<0.001[d]	71.5 (8.7)	65.9 (8.2)	<0.001[d] (0.002[d])

*MS patients *vs* HC; **global heterogeneity among HC, relapse-onset and progressive-onset MS; ***progressive *vs* relapse-onset MS.

[a]Fisher's exact test; [b]Mann-Whitney test; [c]linear model; [d]age- and sex-adjusted linear mixed model accounting for site heterogeneity; [e]age- and sex-adjusted generalized linear mixed model.

^Classification of DMTs: 1st line=interferon beta 1a, glatiramer acetate, teriflunomide and dimethyl fumarate; 2nd line=fingolimod, natalizumab, cladribine, alemtuzumab, rituximab and other immunosuppressants (azatioprine, methotrexate and mitoxantrone).

Abbreviations: IQR=interquartile range; EDSS=Expanded Disability Status Scale; DMT=disease modifying treatment; ARR=annualized relapse rate; LV lesion volume; NBV=normalized brain volume; NcGMV=normalized cortical grey matter volume; NWMV=normalized white matter volume; NDGMV=normalized deep grey matter volume; CSAn=normalized cross-sectional area.

Compared to HC, MS patients were significantly older ($p<0.001$) and had significantly lower NBV, NcGMV, NDGMV, NWMV and cord CSAn ($p<0.001$ for all comparisons). The same variables were different among HC, relapse-onset and progressive-onset MS (Table 1). At *post hoc* analysis, compared to relapse-onset, progressive-onset MS patients were older ($p<0.001$) and more frequently males ($p=0.001$), had higher EDSS score ($p<0.001$), lower annualized relapse rate ($p<0.001$), higher cord lesion number ($p=0.04$) and lower cord CSAn ($p=0.002$). The remaining MRI measures (i.e., T2 LV, T1 LV, NBV, NcGMV, NWMV and NDGMV) did not differ between these two groups.

The median EDSS score was 3.0 at baseline (interquartile range=1.5-5.0) and 3.5 at follow-up (interquartile range=2.0-6.0), with a median EDSS change between baseline and follow-up=0.5 (interquartile range=0.0-1.0, p value *vs* baseline <0.001). During the follow-up, the mean annualized relapse rate was 0.06 (SD=0.16) and 58 MS patients (16%) changed DMT. According to EDSS changes, 120/367 (33%) MS patients showed confirmed EDSS worsening at 5 years; of these, 91 (30%) were relapse-onset MS and 29 (70%) were progressive-onset MS. Thirty-six

relapsing-remitting MS patients (out of 256, 14%) evolved to SPMS. After 5 years, 20 MS patients (out of the 152 MS patients having a baseline EDSS<3.0, 13%) reached EDSS=3.0; 30 MS patients (out of the 209 MS patients having a baseline EDSS<4.0, 14%) reached EDSS=4.0. Finally, 37 MS patients (out of the 288 MS patients having a baseline EDSS<6.0, 13%) reached EDSS=6.0.

Prediction of 5-year outcome.

Clinical worsening. At univariate analysis, baseline candidate predictors (p<0.1) of clinical worsening in all MS patients were older age, higher EDSS score, progressive-onset of the disease, lower NBV, lower NcGMV, higher cord lesion number and a lower cord CSAn (Table 2).

Table 2. Candidate predictors (p<0.10) of disability worsening in patients with multiple sclerosis (MS) (generalized linear mixed-effects models, including follow-up duration, baseline DMT, and DMT change during the follow-up as covariates and random intercepts for site).

Baseline predictor	**OR (95% CI)**	**β**	**p**
Age	1.45 (1.08-1.94)	0.37	0.013
EDSS	1.87 (1.40-2.50)	0.62	<0.001
Type of onset (Progressive- *vs* relapse-onset MS)	3.57 (1.53-8.31)	1.27	0.003
NBV	0.79 (0.60-1.04)	-0.23	0.09
NcGMV	0.66 (0.49-0.88)	-0.42	0.004
Cord lesion number	1.56 (1.19-2.04)	0.44	0.001

Cord CSAn	0.64 (0.49-0.85)	-0.44	0.002

Abbreviations: OR=odds ratio associated with a 1 standard deviation increase; CI=confidence interval; β=standardized beta; EDSS=Expanded Disability Status Scale; NBV=normalized brain volume; NcGMV=normalized cortical grey matter volume; CSAn=normalized cross-sectional area.

The same variables were candidate predictors of clinical worsening also when considering relapse-onset MS patients only. No predictors of clinical worsening were identified by univariate analysis in progressive-onset MS (data not shown).

At multivariate analysis, baseline independent predictors of clinical worsening in all MS patients were progressive-onset (standardized beta [β]=0.97), higher EDSS score (β=0.41), higher cord lesion number (β=0.41), lower NcGMV (β=-0.15) and lower cord CSAn (β=-0.28) (C-index=0.81) (Figure 1). Likewise, in relapse-onset MS the multivariate analysis identified higher EDSS score (β=0.39), higher cord lesion number (β=0.47), lower NcGMV (β=-0.18) and lower cord CSAn (β=-0.35) (C-index=0.79) as independent predictors of 5-year clinical worsening. No predictors of clinical worsening were identified in progressive-onset MS.

Evolution to SPMS. At univariate analysis, baseline candidate predictors ($p<0.1$) of SPMS evolution were male sex, older age, higher EDSS score, longer disease duration, higher brain T2 LV, lower NBV, lower NcGMV, lower NDGMV, and higher cord lesion number (Table 3).

Table 3. Candidate predictors ($p<0.10$) of evolution to secondary progressive (SP) multiple sclerosis (MS) in relapsing-remitting (RR) MS (generalized linear mixed-effects models, including follow-up duration, baseline DMT, and DMT change during the follow-up as covariates and random intercepts for site).

Baseline predictor	**OR (95% CI)**	**β**	**p**

Sex (male *vs* female)	1.94 (0.92-4.10)	0.66	0.08
Age	3.13 (1.90-5.17)	1.14	<0.001
EDSS	5.10 (2.99-8.70)	1.63	<0.001
Disease duration	1.68 (1.07-2.62)	0.51	0.023
T2 LV	1.65 (0.99-2.75)	0.50	0.06
NBV	0.53 (0.34-0.83)	-0.63	0.005
NcGMV	0.52 (0.33-0.82)	-0.65	0.005
NDGMV	0.59 (0.38-0.92)	-0.52	0.02
Cord lesion number	1.83 (1.24-2.70)	0.60	0.002

Abbreviations: OR=odds ratio associated with a 1 standard deviation increase; CI=confidence interval; β=standardized beta; EDSS=Expanded Disability Status Scale; LV=lesion volume; NBV=normalized brain volume; NcGMV=normalized cortical grey matter volume; NDGMV=normalized deep grey matter volume.

At multivariate analysis, older age (β=0.86), higher EDSS score (β=1.40) and higher cord lesion number (β=0.87) independently predicted SPMS evolution (C-index=0.91).

EDSS milestones. At univariate analysis, baseline candidate predictors ($p<0.1$) of EDSS=3.0 milestone were older age, higher EDSS score, lower NBV, lower NcGMV, and higher cord lesion number (Table 4).

Table 4. Candidate predictors (p<0.10) of reaching the three main milestones of Expanded Disability Status Scale (EDSS) score (generalized linear mixed-effects models, including follow-up duration, baseline DMT, and DMT change during the follow-up as covariates and random intercepts for site).

	EDSS=3.0 n*= 20/152 (13%)			**EDSS=4.0** n*=30/209 (14%)			**EDSS=6.0** n*=37/288 (13%)		
Baseline predictor	**OR (95% CI)**	**β**	**p**	**OR (95% CI)**	**β**	**p**	**OR (95% CI)**	**β**	**p**
Age	1.87 (1.04-3.36)	0.63	0.037	3.12 (1.82-5.35)	1.14	<0.001	2.62 (1.64-4.19)	0.96	<0.001
EDSS	4.70 (2.09-10.59)	1.55	<0.001	11.47 (4.46-29.49)	2.44	<0.001	6.12 (3.42-10.95)	1.81	<0.001
NBV	0.62 (0.35-1.07)	-0.49	0.08	-	-	-	-	-	-
NcGMV	0.58 (0.32-1.04)	-0.55	0.07	0.54 (0.34-0.87)	-0.61	0.011	0.55 (0.36-0.83)	-0.60	0.005
Cord lesion number	1.57 (0.97-2.56)	0.45	0.07	1.63 (1.07-2.47)	0.49	0.023	1.88 (1.26-2.82)	0.63	0.002
Cord CSAn	-	-	-	0.49 (0.31-0.79)	-0.70	0.003	0.62 (0.42-0.92)	-0.48	0.017

*number (percentage) of MS patients reaching the milestone at 5 years.

Abbreviations: OR=odds ratio associated with a 1 standard deviation increase; CI=confidence interval; β=standardized beta; NBV=normalized brain volume; NcGMV=normalized cortical grey matter volume; CSAn=normalized cross-sectional area.

Baseline univariate candidate predictors ($p<0.1$) of EDSS=4.0 and 6.0 were older age, higher EDSS score, lower NcGMV, higher cord lesion number and lower cord CSAn (Table 4). At multivariate analysis, baseline predictors of reaching EDSS=3.0 milestone were higher EDSS score (β=1.49), higher cervical cord lesion number (β=1.02) and lower NcGMV (β=-0.56) (C-index=0.88, Figure 2). Baseline older age (β=0.30), higher EDSS score (β=2.03), higher cervical cord lesion number (β=0.66), and lower cord CSAn (β=-0.41) were independent predictors of reaching EDSS=4.0 milestone (C-index=0.92, Figure 2). Finally, higher baseline EDSS score (β=1.87) and higher cervical cord lesion number (β=0.54) independently predicted reaching EDSS=6.0 milestone (C-index=0.91, Figure 2).

DISCUSSION

Here, we investigated the predictors of clinical progression after 5.1 years of a large cohort of definite MS patients, and found that a combination of clinical, brain and cervical cord MRI variables predict clinically relevant neurological outcomes at follow-up. Of note, higher EDSS and higher cord lesion number independently predicted all neurological outcomes (i.e., EDSS worsening, SPMS evolution, EDSS milestones). In the whole MS cohort and in relapse-onset MS, multiparametric measures (lesions and tissue loss) of brain and spinal cord damage independently predicted 5-year disability worsening. In addition, cortical volume loss was associated with reaching an EDSS=3.0, while lower cord area was one of the determinants of EDSS=4.0.

Consistently with previous studies,[6, 9, 10, 14, 17, 19] clinical worsening was evaluated on 3-month confirmed changes of the EDSS score, one of the most widely used disability scales. We also selected evolution to SPMS as a clinically relevant outcome. Finally, EDSS=3.0, 4.0 and 6.0 represent crucial disability points for disease worsening. During the 5-year follow-up, 33% of patients had a worsening of disability and 14% of relapse-onset MS evolved to SPMS. Of note, 30% of relapse-onset MS and 70% of progressive-onset MS were clinically worsened: these rates

are in line with previous findings[10, 15] and reflect the identification of progressive- *vs* relapse-onset MS as an independent predictor of clinical worsening at multivariate analysis. The importance of baseline EDSS score for subsequent clinical outcomes is in line with several previous reports[8, 17, 30, 31] and reinforces the notion that this variable needs to be taken into consideration for MS management.[30] On the other hand, since defining clinical worsening is based on the EDSS score itself, this result might be partially explained by autocorrelation.

Besides baseline EDSS and a progressive-onset, three MRI measures contributed to the prediction of 5-year clinical worsening in multivariate models, namely, higher cord lesion count, lower NcGMV and lower cervical cord CSAn. The identification of cord lesions as a predictor of 5-year clinical worsening is in line with their relevance in MS prognosis at early disease stages.[4, 11] In earlier studies, spinal cord lesions showed modest correlations with disability in clinically definite MS.[32] Availability of 3.0T scanners and improved spinal cord MRI acquisition allowed a better cord lesion detection, resulting in increased correlations with clinical measures.[33] In line with this, recent studies reported a significant predictive role of cervical cord lesions on subsequent MS disease course.[5, 9]

Among brain MRI variables, the only independent contributor was GM volume loss, which has a well-established role in predicting medium- and long-term MS disease progression.[10, 13-15, 17, 20] This reinforces the notion that, in addition to inflammation and demyelination, neurodegeneration significantly contributes to MS course. Interestingly, a lower cord CSAn contributed independently to predict clinical worsening, suggesting that compartmentalized CNS atrophy are major risk factors for disease progression.[18, 21] Also, the presence of both spinal cord lesions and cord CSAn in the final model suggests a strong role of cord damage in explaining 5-year clinical worsening.

Interestingly, higher baseline EDSS score and cord lesion count, as well as lower NcGMV and cervical cord CSAn, were significant predictors of 5-year clinical worsening not only in all MS patients, but also in relapse-onset MS. This reinforces the importance of baseline disability, brain and cord MRI damage, in explaining subsequent clinical deterioration in MS patients having the

most typical disease onset. No significant predictors of clinical worsening were found in progressive-onset MS. This is likely due to the relatively small sample size of this group combined with a plateauing effect of EDSS and MRI-detected damage, which were already high at baseline visit.

When looking at independent predictors of SPMS evolution, an older age and baseline EDSS scores were the two demographic/clinical variables retained in the multivariate model. This confirms findings from previous epidemiological studies[31, 34] and supports the notion that age is an important risk factor for SPMS evolution. Indeed, about 50% of relapsing-remitting MS convert to SPMS within 10-15 years, and about 90% within 25 years if untreated.[31, 34] The only MRI variable associated with SPMS evolution at multivariate analysis was cervical cord lesion number. This is not surprising, given the crucial role of spinal cord imaging features in diagnosing progressive MS from MRI.[35] In SPMS, increase of disability is mostly driven by ambulation impairment. As such, a key role of spinal cord damage in explaining conversion to SPMS is not unexpected. Also, a larger extent of cervical cord lesions in SPMS *vs* relapsing-remitting MS was frequently reported.[32, 35] Disappointingly, CSAn was not identified as an independent predictor of SPMS evolution. This seems to contradict previous studies evidencing a role of accelerated cord atrophy in explaining subsequent SPMS conversion.[25] However, previous findings were mainly based on temporal trajectories of damage accumulation,[21, 25] which might be more clinically relevant than MRI measurements at a single time point. Unfortunately, a 1-year follow-up MRI evaluation (described in details elsewhere)[26] was available in less than 50% of our sample, not allowing this type of analysis. Moreover, a plateauing effect of cord atrophy might be also present. Finally, whole-cervical CSAn might be mainly sensitive to upper cord injury, while damage to lower cervical[26] or thoracic[33] cord might be more relevant in severely disabled MS patients.

Models assessing the main determinants of EDSS milestones at 5-years also revealed interesting results. Besides spinal cord lesion number and baseline EDSS score, a lower NcGMV predicted reaching an EDSS=3.0, while cervical cord CSAn predicted an EDSS=4.0. Remarkably,

both these MRI metrics are related to CNS atrophy, while T2 LV was never selected as independent predictor of any milestone. This suggests that neurodegeneration is more important than focal demyelination to explain the accumulation of clinical disability and that cortical volume loss is a silent predictor of concomitant and future clinical disability in MS.[36, 37] With regards to EDSS=3.0, a previous cross-sectional study already found that brain GM volume loss was relevant to explain such milestone.[38] Here, volume loss of this compartment was the only one having a significant role in predicting subsequent disability, while other studies evidenced some contribution also of deep GM.[13, 16] However, these studies did not perform *ad hoc* analyses for specific milestones. It might be therefore likely that reduced deep GM volume, being present in a wide proportion of MS patients from disease beginning, constitutes a global worse prognostic factor, but it is not specific enough to differentiate subjects reaching an EDSS=3.0 from those remaining below 3.0 over a 5-year period. The milestone of EDSS=4.0 is particularly relevant in MS, because it is related to locomotor disability. Here, we found that such milestone was predicted by a lower cord CSAn (besides a higher cervical cord lesion count). This is not surprising, since this milestone characterizes MS patients with a moderate disability mainly driven by locomotor impairment. Moreover, a high degree of cord atrophy was already demonstrated in patients with *vs* those without locomotor impairment.[39] The cervical cord incorporates all descending corticospinal fibers directed to motor units. Therefore, it is conceivable that cord damage has a disproportionately severe effect on locomotor functions. Finally, the only MRI predictor of EDSS=6.0 was cervical cord lesion count. Once again, this confirms the high relevance of focal cord demyelination in the generation of severe clinical disability[9, 38] and reinforces the notion that, once spinal cord involvement becomes extensive, it overrides contributions by other CNS compartments.[38] Once again, CSAn was probably not retained as independent predictor of EDSS=6.0 due to plateauing effects of cord atrophy and clinical relevance of lower cord damage in severely disabled patients.

This study is not without limitations. First, we had no clinical measures of upper limb and cognitive impairment of our patients. This may lead to some bias in our results towards weighting

locomotor disability, strongly linked to the EDSS score. Investigating correlations with deterioration of specific motor scales or cognitive worsening might be useful to give a more complete picture of the mechanisms underlying disease progression in MS. Second, baseline MRI included only conventional MRI sequences for lesion and atrophy quantification. The lack of axial T2-weighted cord scans with a relatively high in-plane resolution made impossible a reliable estimation of cord lesion volume and lesion location. Moreover, the use of advanced MRI techniques (e.g., diffusion-weighted MRI and functional MRI) could help to estimate the relative contribution of WM injury (in particular, of the corticospinal tract) and functional plasticity on disability progression. However, the measures investigated here are easily obtainable and offer an immediate clinical applicability. Finally, from a technical point of view, it is also important to note that cord lesion count and CSAn were derived from a previous study[26] using manual and semi-automatic evaluation methods. The recent introduction of fully automated methods of cord lesion detection (based on convolutional neural networks) and cord area quantification (as those included in the Spinal cord toolbox)[40] may increase operator-independence and analysis speed, and may facilitate the translation of such methods to non-specialized neuroimaging sites. Third, no intermediate clinical assessments were available for enrolled patients. Therefore, it was not possible to exactly define the moment when patients evolved to SPMS and perform time-dependent analyses. Fourth, an independent cohort for validating identified associations was not available. Finally, no images of other cord portions were obtained.

To conclude, in MS patients, baseline spinal cord lesions, cord tissue loss and cortical volume loss independently predicted 5-year disability worsening, reaching of EDSS milestones and SPMS conversion. The combined assessment of brain and spinal cord damage may contribute to identify MS patients having long-term disease progression, and the introduction of automated analysis methods for cord lesion and atrophy quantification may expedite a wider use of these metrics to clinical practice.

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AUTHOR CONTRIBUTIONS

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COMPETING INTERESTS

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DATA SHARING

The dataset used and analyzed during the current study are available from the corresponding author on reasonable request.

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FIGURE LEGENDS

Figure 1. Independent predictors of clinical disability worsening at multivariate analysis. A) The figure illustrates the variable selection procedure of the generalized linear mixed model with L1-penalized estimation for clinical disability worsening in all patients with multiple sclerosis (MS). The plot shows the profile of standardized beta coefficients against the decreasing values of the tuning parameter λ. The vertical dashed line indicates the optimal value of the tuning parameter λ, which minimizes the Akaike Information Criterion (AIC), at which non-zero coefficients are obtained for type of onset (progressive- *vs* relapse-onset MS) (violet), baseline EDSS score (green), cord lesion number (red), NcGMV (orange) and cord CSAn (blue). The path of coefficients for not selected predictors are drawn in grey. B) ROC curve for the final model for clinical disability worsening, including only the selected features.
Abbreviations: EDSS=Expanded Disability Status Scale; NcGMV=normalized cortical grey matter volume; CSAn=normalized cross-sectional area.

Figure 2. Independent predictors of clinical disability milestones at multivariate analysis. Illustrative representation of demographic, clinical and MRI predictors, selected with the generalized linear mixed model with L1-penalized estimation, contributing to reaching different disability milestones at 5 years in patients with multiple sclerosis (MS).
Abbreviations: EDSS=Expanded Disability Status Scale; NcGMV=normalized cortical grey matter volume; CSAn=normalized cross-sectional area.