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ORIGINAL COMMUNICATION

Brain tissue volumes and relaxation rates in multiple sclerosis: implications for cognitive impairment

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Abstract

Objective Both normal gray matter atrophy and brain tissue relaxation rates, in addition to total lesion volume, have shown significant correlations with cognitive test scores in multiple sclerosis (MS). Aim of the study was to assess the relative contributions of macro- and microstructural changes of both normal and abnormal brain tissues, probed, respectively, by their volumes and relaxation rates, to the cognitive status and physical disability of MS patients.

Methods MRI studies from 241 patients with relapsing–remitting MS were retrospectively analyzed by fully automated multiparametric relaxometric segmentation. Ordinal backward regression analysis was applied to the resulting volumes and relaxation rates of both normal (gray matter, normal-appearing white matter and CSF) and abnormal (T2-weighted lesions) brain tissues, controlling for age, sex and disease duration, to identify the main independent contributors to the cognitive status, as measured by the percentage of failed tests at a cognitive test battery (Rao's Brief Repeatable Battery and Stroop test, available in 186 patients), and to the physical disability, as assessed by the Expanded Disability Status Scale (EDSS).

Results The R1 relaxation rate (a putative marker of tissue disruption) of the MS lesions appeared the single most significant contributor to cognitive impairment ($p < 0.001$). On the contrary, the EDSS appeared mainly affected by the decrease in R2 of the gray matter ($p < 0.0001$), (possibly influenced by cortical plaques, edema and inflammation).

Conclusions In RR-MS the tissue damage in white matter lesions appears the single main determinant of the cognitive status of patients, likely through disconnection phenomena, while the physical disability appears related to the involvement of gray matter.

Keywords Atrophy · Multiple sclerosis · Quantitative MRI · Relapsing/remitting · Cognitive impairment · Relaxation rates

Introduction

Cognitive impairment represents a well-known feature of multiple sclerosis (MS) [1], and has shown in previous studies a complex pattern of correlations with MRI-derived quantitative measures [2].

In particular, while total lesion volume has shown limited correlation with cognitive test scores in most previous studies [2], gray matter (GM) atrophy has been fairly consistently related to cognitive performance in MS [3, 4], and a few cross-sectional studies directly comparing these measures suggest that brain volume loss may be more strictly related to cognitive impairment than lesion load [5–7].

In addition to volumetric measures, other quantitative MRI-derived parameters, such as longitudinal (T1) and transverse (T2) relaxation times, provide additional

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information on tissue integrity [8], and are simultaneously influenced by the combined effects of the different pathological substrates of MS, including edema, de- and re-myelination, axonal loss, and gliosis. In particular, although no single MRI-derived measure is specific for a distinct component of the pathological process underlying MS pathology, T2 relaxometry is mainly influenced by tissue myelin content [8], edema [9] and inflammation [10], while T1 relaxation times are related mainly (but not only) to the microstructural tissue integrity, including myelin content and axonal density [11]. Accordingly, thanks to their different sensitivities to the same pathological substrates, T1 and T2 relaxation rates have the potential to amplify the underlying tissue-related information while limiting the noise [12–14], so that these measure appear of interest for quantification of both brain tissue damage [15–17] and axonal loss within WM lesions [18].

Not surprisingly, the relaxometric properties of both lesions [19–21] and normal-appearing white matter (NAWM) [18] have shown significant correlations to the cognitive performance of MS patients. However, to date only few studies [19, 22, 23] have assessed the relative merits of the relaxometric and volumetric measures in defining the clinical status, suggesting that global cortical GM relaxation rates may correlate better than simple volumetric measures with information processing speed [19, 22].

Aim of the present study was to assess the relative contributions to the cognitive status and physical disability of both macro- and microstructural integrity of normal-appearing brain tissues and white matter lesions in MS patients. To this end, we tested the correlations of the volumes and relaxation rates of GM, NAWM, CSF and abnormal white matter (aWM), measured by multiparametric relaxometric segmentation of clinical MRI studies, with the results of a cognitive test battery and with the Expanded Disability Status Scale (EDSS) scores, assessing their relative independence through stepwise regression analysis.

Methods

Patients

Medical records of patients with clinically definite relapsing–remitting MS according to the McDonald criteria [24], who had undergone MRI studies suitable for relaxometric segmentation (see below for sequence parameters) as part of their participation in clinical trials or for routine clinical follow-up at the MS Center of the University "Federico II" of Naples, were reviewed.

We considered only MRI studies of patients for whom the EDSS and/or the cognitive test battery routinely administered in the MS Center (see below) had been collected within 1 month from the MRI.

Table 1 Correction factors for scores

Test	Correction factors	Threshold
SRT-LTS	Education (1.402/year)	23.3
SRT-CLT	Education (1.542/year)	15.5
SRT-D	Education (0.201/year)	4.9
SPART	Education (0.368/year)	12.7
SPART-D	Education (0.128/year)	3.6
SDMT	Education (1.029/year)	37.9
PASAT 2	Education (1.116/year)	17.1
PASAT 3	Education (1.698/year)	28.4
WLG	Sex (male + 2.123, female − 2.123)	17.0
Stroop	Age (0.28/year), sex (male + 1.70, female − 1.70), education (4.09/year)	11.0

Factors employed for correction of scores from cognitive tests, and corresponding thresholds for binarization. Correction factors and normative data are derived from by Barbarotto et al. [26] for the Stroop test, from Amato et al. [27] elsewhere

Exclusion criteria were: age < 18 years; presence of other clinically significant neurological or vascular diseases, psychiatric conditions; previous therapies with monoclonal antibodies, cytotoxic or immunosuppressive therapy (excluding systemic steroids and/or ACTH).

Clinical and neuropsychological assessment

Cognitive tests considered for the present study, administered by trained neuropsychologists, included Rao's Brief Repeatable Battery (BRB) [25] and the Stroop test [26].

The BRB includes: the Selective Reminding Test, a measure of both verbal learning and long-term memory, providing scores for long-term storage (SRT-LTS), consistent long-term retrieval (SRT-CLTR) and delayed recall (SRT-D); the 10/36 Spatial Recall Test, assessing both immediate (SPART score) and delayed (SPART-D) visuospatial learning; the Symbol Digit Modalities Test (SDMT, a measure of information processing speed); the Paced Auditory Serial Addition Test (PASAT, a measure of sustained attention and speed of information processing); the Word List Generation (WLG, a semantic fluency task).

In addition, the Stroop test (ST) was included, which evaluates both sustained attention and the ability to inhibit automatic responses while performing a task based on conflicting stimuli. For the present study, the time required to process 50 items when asked to name the color of the ink of words indicating conflicting colors was used.

The raw scores of the Rao's brief battery were adjusted for sex (the WLG scores) and for education (all other scores), according to the normative study by Amato et al.

[27], from which the "Version A" parameters were used (reported in Table 1).

The Stroop test scores were analogously corrected for age, sex and education according to the "card presentation" version of the test reported by Barbarotto et al. [26] (Table 1).

Finally, resulting corrected scores were binarized using as threshold the fifth percentile (below 2 SD from the mean) of the corresponding normative datasets (Table 1) [27], and a global cognitive index (CI) was defined as the relative proportion of impaired cognitive tests.

MRI

MRI studies were carried out at 1.5 T (Intera, Philips Medical Systems, the Netherlands), and included two conventional spin-echo sequences providing T1w and PD/T2w contiguous axial images covering the whole brain. Suitable acquisition protocols included four different protocols which have been used over time (Table 2).

In particular, acquisition protocols A and B had been used within two previous studies on the efficacy of atorvastatin as add-on therapy to interferon [28, 29] (from which the baseline MRI scans have been used here), while protocols C and D had been used in routine clinical studies (the only difference between protocols C and D being their dual-echo sequences, Table 2).

For each study, T1w and PD/T2w volumes were preliminarily co-registered using the automated rigid body co-registration routine available in Statistical Parametric Mapping software (SPM8, http://www.fil.ion.ucl.ac.uk/spm/), to remove potential misalignment resulting from between-sequence patient movements. For co-registration, normalized mutual information was used as objective function, and SPM8 default parameters were used.

The co-registered MRI volumes were segmented into GM (including both the cortex and deep GM structures), NAWM, abnormal WM (aWM), and CSF, using a fully automated relaxometric method described in detail elsewhere [30, 31]. Shortly, from the triplet of T1-, proton density- and T2-weighted images the segmentation algorithm calculates for each voxel the R1 (equal to 1/T1) and R2 (1/T2) (the relaxation rate calculation procedure is detailed in the supplementary material). Proton density, R1 and R2 maps are then merged into a single color image [32] by coding, respectively, its red, green and blue channels (Fig. 1, upper row) to allow visual assessment of image quality, and automatically segmented into normal-appearing GM, NAWM, aWM and CSF (Fig. 1, bottom row), based on the relaxometric properties of each voxel. The segmentation is achieved preliminarily assigning the voxels with relaxation rates "typical" for a specific tissues to the corresponding map, while cluster of voxels with relaxometric properties intermediate between different tissues are assigned to a specific tissue based on a combined probability approach, which takes into account also on the composition of the surroundings. From the relaxation rate maps and the segmented maps, the volume and the mean relaxation rates of each tissue are then calculated.

Table 2 MRI protocols

	Protocol A ($n=60$)	Protocol B ($n=86$)	Protocol C ($n=42$)	Protocol D ($n=53$)
T1-weighted				
TR (ms)	600	520	500	500
TE (ms)	15	15	10	10
WFS (pixels)	2	2	1.2	1.2
Number of interleaved series	2	2	3	3
Number of averages	2	2	2	2
Acquisition duration	7′44″	5′48″	9′40″	9′40″
PD-/T2-weighted				
TR (ms)	2200	1800	2300	2300
TE1/TE2 (ms)	15/90	15/90	10/80	10/80
WFS (pixels)	2	2	0.7	1.2
Number of interleaved series	2	2	2	2
Number of averages	1	1	1	1
Acquisition duration	14′09″	9′42″	12′11″	12′11″
Voxel size (mm^3)	0.94×0.94×4	0.86×0.86×4	0.98×0.98×3	0.98×0.98×3
Number of slices	32	32	48	48

Conventional spin-echo sequences used for MRI relaxometric brain tissue segmentation

WFS water–fat shift

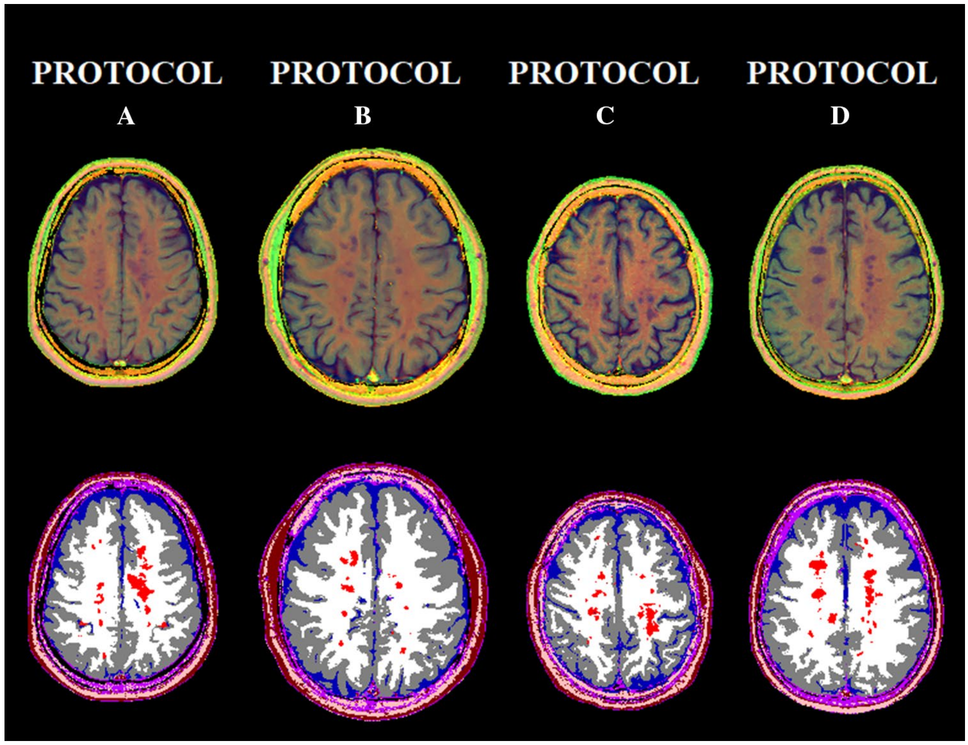

Fig. 1 Representative slices at the level of centra semiovalia from four patients with the four acquisition protocols used in the present study. Multiparametric color-coded images (in which red, green and blue image channels are coded by the proton density, R1 and R2 maps, respectively) are displayed (upper row) along with the corresponding segmented maps (bottom row). In the segmented images, gray matter is shown in gray, white matter in white, CSF in blue, aWM in red

Of note, the segmentation method used for the present study does not require preliminary lesion-filling procedures, as a previous validation study has shown a lack of a aWM-associated bias in the measure of brain tissue volumes [33].

To take into account head size, for each subject, each tissue volume was preliminarily divided by the total intracranial volume (ICV, the sum of the voxels belonging to all intracranial tissues and CSF), providing corresponding fractional volumes (fCSF, fGM fNAWM and faWM). Note that fCSF is complementary to the frequently used brain parenchyma fraction (also called normalized brain volume, the sum of GM, aWM and NAWM), so that their use in the context of the statistical analyses below is equivalent.

In most patients, T1-weighted sequences repeated at least 5′ after the i.v. injection of a 0.1 mmol/kg gadopentetate dimeglumine—Magnevist, or gadobutrol—Gadovist (Bayer Schering Pharma AG, Berlin, Germany), to assess ongoing inflammatory disease activity were also acquired.

Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Science (SPSS) package (v. 15.0, SPSS Inc., Chicago, USA).

Due to the non-parametricity of EDSS, and non-normality of both outcome scores ($p < 10^{-10}$ at Kolmogorov–Smirnov normality test), correlations with EDSS and CI of brain tissue fractional volumes and relaxation rates were tested by backward ordinal regression analysis (using the Polytomous Logit Universal Models—PLUM—procedure in SPSS), to define the most important predictors of these two clinical scores, forcing first MRI acquisition protocol, age, sex and disease duration (DD) in the model.

As an ancillary analysis, the same procedure was carried out adding in the first step also the presence of contrast-enhancing lesions, to rule out the possible effect of ongoing inflammatory activity on the correlations.

Results

Two hundred forty-one patients (146 under treatment with IFN-β) fulfilled inclusion and exclusion criteria, including 186 patients for whom cognitive data were available. Demographic, clinical and cognitive data are summarized in Table 3.

In 228 patients, post-contrast T1-weighted sequences were available. Of these, 57 showed contrast-enhancing lesions.

At ordinal regression analysis (Table 4), EDSS appeared essentially related to GM-R2 (slope -0.191, $p \leq 10^{-4}$), with a trend for correlation with CSF increase, WM decrease and R2 of normal-appearing white matter ($p < 0.05$ for all). On the other hand, CI correlated with almost all the tested MRI-derived measures, with the exception of the fGM and aWM-R2.

Table 3 Cognitive test results

	n	Mean	SD	Min	Max	Failures
Age	186	34.1	8.0	18	54	
Sex (F/M)		112/74				
EDSS		2.4	0.8	1.0	5.0	
DD (years)		4.1	5.0	0.1	24.3	
PASAT 2	159	27.7	12.1	7	67	27 (17.0%)
PASAT 3	171	35.4	13.3	5	60	51 (29.8%)
SDMT	143	48.4	14.6	14	94	29 (20.3%)
SRT-CLT	184	27.4	12.3	3	59	29 (15.8%)
SRT-D	185	7.3	2.4	2	12	34 (18.4%)
SRT-LTS	186	37.9	12.3	4	64	16 (8.6%)
STROOP	186	19.9	7.0	3	37	55 (29.6%)
WLG	143	30.7	12.1	7	66	22 (15.4%)
SPART	185	7.3	2.2	1	10	10 (5.4%)
SPART-D	185	20.9	5.0	8	30	7 (3.8%)
CI	186	16.6%	17.5%	0.0%	80.0%	

Failures: number of tests scoring > 2SDs below the corresponding mean normative values

CI cognitive index

Table 4 Results of ordinal regression analyses

	Main analysis (age, sex and DD as covariates)				Ancillary analysis (age, sex, DD and enhancing lesions as covariates)			
	EDSS ($n=241$)		CI ($n=186$)		EDSS ($n=228$)		CI ($n=179$)	
	Slope	p	Slope	p	Slope	p	Slope	p
fGM	–	n/s	–	n/s	–	n/s	–	n/s
fNAWM	−0.101	0.015	−0.148	0.002	−0.113	0.009	−0.128	0.009
fWM	−0.121	0.013	−0.150	0.008	−0.137	0.007	−0.121	0.039
fCSF	0.090	0.013	0.124	0.004	0.095	0.011	0.110	0.012
faWM	–	n/s	0.321	0.019	–	n/s	0.336	0.019
GM-R1	–	n/s	−9.701	0.002	–	n/s	−9.407	0.003
GM-R2	**−1.571**	**$<10^{-4}$**	−1.367	0.002	**−1.585**	**$<5\times10^{-4}$**	−1.287	0.005
NAWM-R1	–	n/s	−6.396	0.002	–	n/s	−6.421	0.002
NAWM-R2	−0.950	0.011	−1.175	0.006	−0.835	0.033	−1.114	0.013
WM-R1	–	n/s	−6.364	0.002	–	n/s	−6.399	0.002
WM-R2	−0.766	0.012	−1.060	0.003	−0.710	0.027	−1.032	0.005
aWM-R1	–	n/s	**−6.003**	**0.001**	–	n/s	**−5.688**	**0.001**
aWM-R2	–	n/s	–	n/s	–	n/s	–	n/s

Significant correlations of EDSS and cognitive index (CI, the percentage of failed cognitive tests) with brain tissue volumes and relaxation rates. Age sex and DD are entered in a preliminary step. Regression slopes (in s^{-1}) and corresponding uncorrected p values for the effect of each MRI-derived measure assessed independently are reported. Variables retained at backward regression analyses are in bold. The main analysis has been replicated including also the presence of enhancing lesions among the nuisance covariates (ancillary analysis), to rule out the effect of disease activity on the correlations

n/s non-significant

At backward regression analysis, the only determinants retained in the model were GM-R2 for EDSS, and aWM-R1 (slope −0.059, $p=0.001$) for CI.

The ancillary analysis confirmed that these patterns of correlations were not influenced by disease activity.

Discussion

The present results highlight a preferential correlation, in MS, of the cognitive status with the relaxometric properties (in particular with the R1) of the WM lesions, while physical disability appears influenced mainly by GM involvement.

Results for cognitive status are in line with previous findings obtained sampling at higher resolution brain tissue T1 and volumes, a possibility provided by the recent introduction of 3D sequences allowing T1 relaxometry with small isotropic voxels. In particular, previous studies using the DESPOT1-HIFI sequence in MS patients with mixed disease courses [19], and the MP2RAGE sequence in a group of RR-MS patients [20] regression analyses have consistently identified the T1, rather than the volume, of WM lesions among the main determinants of CI.

Cognitive impairment in MS is characterized by a pattern similar to the so-called subcortical dementia, which is consistent with a disconnection syndrome [34], suggesting that in MS the widespread WM damage may hamper the functional connections among different cortical areas and with deep GM structures, resulting in cognitive deficits. This hypothesis is partly supported by the correlation between T2-weighted lesion load and cognitive performance that has been demonstrated in several studies (a trend also present in the current data, although not retained by the backward regression procedure). However, the limited specificity of T2-weighted lesion load (which includes MS lesions with pathological substrates ranging from inflammation and edema to axonal loss [35]) results in a need of additional measures that assess the severity of damage within MS lesions.

Low R1 values are indeed typical of black holes, more destructive lesions associated to axonal loss and transection [35]. Accordingly, similar to what has been proposed for MTR [36] or diffusion parameters [37], aWM-R1 may represent a marker of the severity of the lesions, resulting in substantial disconnection.

This result is indeed also in line with the preferential correlation of T1-weighted lesion load increase with cognitive impairment, confirmed by previous longitudinal studies [38].

Contrary to some previous studies [3, 4], we did not find a significant correlation between fGM volume and CI. Besides possible differences in patient populations, this difference may be partly explained by methodological differences, as in our case a multiparametric relaxometric segmentation method was used, applied to 2D-SE sequences, as opposed to the monoparametric segmentation of T1-weighted isotropic volumes used in previous studies.

It is possible that the limited spatial resolution of the sequences processed in the present work may have penalized the sensitivity of our volume measurements, compared to isotropic 3D T1-weighted volumes analyzed in most studies of atrophy in MS, although the strong correlations that were found with the relaxation rates, measured at the same resolution, mitigate against this hypothesis.

On the other hand, it may be speculated that the presence of changes in T1 relaxation time of normal-appearing GM, which are present in MS [19], may affect the GM segmentation when using a monoparametric method. This has the potential to emphasize correlations of fGM with the cognitive status, as GM-R1 appeared to correlate with CI in our data, consistent with the correlation of the skewness of the GM T1 histogram with the cognitive status [19].

GM-R2, selected by the backward regression analysis as main determinant of EDSS, may in principle reflect the effect of several different, and likely intermingled, phenomena, ranging from changes in tissue myelin content [8], to edema [9] and inflammation [10], coupled do the possible presence of cortical lesions (known to correlate with both physical and cognitive impairments [4]).

The persistence of this finding in the ancillary analysis, however, indicates that edema and ongoing active inflammation are unlikely to play a major part in determining this correlation, as the inclusion in the model of the presence of enhancing lesions (an indicator of BBB breakdown, and hence of active inflammatory phenomena) did not affect this correlation.

In addition, partial volume effects by CSF, due to sulcal enlargements occurring with increasing cortical atrophy, may also influence GM-R2, especially considering the significant slice thickness of the clinical studies analyzed. However, the limited correlation between EDSS and fCSF in our data suggests that this mechanism is unlikely to justify this correlation in this case. Further studies exploiting relaxometric measures based on isotropic 3D acquisitions [39], and including sequences suitable for cortical lesion segmentation are needed to fully address these issues.

Finally, some limitations of the present study should be considered.

In the present work, calculation of the relaxation rates was based on a monoexponential decay model, which however represents an approximation of the actual multiexponential relaxation parameters in the CNS tissues, which may have relevant implications to the study of multiple sclerosis pathophysiology [40, 41].

Indeed the TEs of the double-echo sequences that were employed in this work fall in the range of the T2 of myelin water (approximately 20 ms) and free water (approximately 80 ms) fractions [41], so that changes in calculated R2s can be induced both by a change in the relative percentages of these two water components, and/or by changes in their relaxation rates. Further studies, using multi-echo sequences for multiexponential decay curve fitting, which however are not currently compatible with clinical routine, are needed to evaluate whether the separate assessment of these two compartments may provide additional information.

The tissue volume and relaxation rate maps used for the present study are calculated on 2D conventional spin-echo images. While this allowed to collect retrospectively data from a large patient group, the limited axial resolution of the sequences results in significant partial volume effects, which are especially relevant for GM, considering the limited thickness of the cortical ribbon. Additional studies, using isotropic 3D-sequences, are needed to rule out the effect of the limited resolution on current results.

Eventually, for the present work only whole-brain values have been considered, although heterogeneity across the brain of both atrophy [31] and microstructural tissue changes [42] appears to play a role in both physical disability and cognitive impairment. Accordingly, ROI- or voxel-based analyses should be undertaken, that may help to further clarify the mechanisms underlying the relationship of these volumetric and microstructural alterations with the clinical features of the disease.

Conclusion

Using a fully automated relaxometric segmentation method, based on clinical conventional spin-echo sequences, we have shown in a large group of relapsing–remitting MS patients a preferential correlation of the cognitive status with the microstructural properties of the WM lesions, as assessed by R1, a putative marker of tissue disruption.

Physical disability, on the other hand, had in GM-R2 its main determinant, possibly related to the presence of cortical lesions and/or microstructural alterations.

These correlations, which are independent of disease duration or the presence of enhancing lesions, highlight the role of quantitative MRI measures in assessing clinic–radiologic correlations in MS, providing additional information on the pathophysiological substrates of cognitive impairment in MS.

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Compliance with ethical standards

Ethical standards All human studies have been approved by the Institutional Review Board of the university "Federico II" and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent All participants gave informed written consent.

Conflicts of interest R.L. received personal fees for public speaking or consultancy from Merck, Novartis, Biogen, Genzyme, Teva and Almirall. V.B.M. received personal fees for public speaking or consultancy from Bayer, Mylan, Merck, Novartis, Biogen, Genzyme, Teva and Almirall. M.M. declares that he has received honoraria and support for travelling from Almirall, Coloplast, Genzyme and Merck Serono. R.M., B.A., T.C., M.C., G.V., A.C., A.P., G.S., and M.Q. declare that they have no conflict of interest.

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