

## **Abstract**

**Background:** Brain atrophy in multiple sclerosis (MS) patients is present since the very early stages of the disease and it has been related to long-term disability.

**Objective:** To estimate brain volume (BV) at 15 years after a clinically isolated syndrome (CIS) and to evaluate its relationship with disease outcomes.

**Methods:** From a prospective cohort including patients presenting with a CIS, 54 patients with a brain MRI performed 15 years after CIS were included. Brain parenchymal fraction (BPF), grey matter fraction (GMF) and white matter fraction (WMF) at 15-year follow-up were obtained. Regression analyses were conducted to predict BV loss and reaching an EDSS of 3.0 in that 15-year period.

**Results:** In multivariable analyses, lower values of BPF and WMF were significantly associated with being male, presenting 3-4 Barkhof criteria at baseline, presenting a second relapse, and with a decision to start treatment. In the multivariable logistic regression analysis, only lower GMF was associated with a greater risk of reaching EDSS 3.0 (OR 0.24,  $p=0.028$ ).

**Conclusion:** Lower BPF and WMF 15 years after CIS are associated with previous markers of inflammatory disease. Lower GMF 15 years after a CIS is associated with an increased risk of reaching an EDSS of 3.0.

## Introduction

Multiple sclerosis (MS) pathogenesis involves different processes of demyelination, gliosis, and axonal damage that may ultimately lead to irreversible tissue destruction and the development of brain atrophy [1]. Brain volume (BV) loss in MS patients can be measured with automatic, precise, and reproducible software [2].

Using these tools, it has been demonstrated that BV loss in MS patients occurs at a rate of 0.5% to 1% per year [3]–[5], which is higher than in healthy controls [5]. The rate of BV loss is similar in all stages of the disease [4] and is present very early after disease onset [6]–[9]. In fact, the development of BV loss, and specially grey matter (GM) volume loss, short after presenting a clinically isolated syndrome (CIS) has been associated with a higher risk of developing MS during the follow-up [6]–[9]. Moreover, global BV loss, and specially GM volume loss, has been related with the extent of disability as measured with the Expanded Disability Status Scale (EDSS) at one year, five years, and 20 years after a CIS [8],[10]–[12]. Despite BV loss being such a relevant measure for MS patients, only one study assessed long-term brain atrophy after a CIS [12], and none has evaluated its relationship with baseline clinico-radiological variables.

The aims of this work were: (1) to analyse global and tissue-specific BV loss 15 years after a CIS, (2) to investigate the impact of baseline and follow-up clinical, radiological, and demographical variables on the development of global and tissue-specific BV loss 15 years after CIS onset, and (3) to study the relationship between global and tissue-specific BV loss 15 years after CIS onset and the development of neurological disability.

## Material and Methods

*Patients.* The present work is based on a prospective on-going cohort including patients presenting with a CIS that has been recently described [13]. From this cohort, patients with a brain MRI performed 15 years after a CIS were included. At baseline, demographical, clinical, and radiological data were recorded. Clinical assessments were conducted on a regular basis (every 6 to 12 months); in each visit the presence of new relapses and the EDSS score was collected. This study received approval from the local ethical committee, and all patients signed a written informed consent form.

*MRI acquisition and analysis.* Brain MRI was performed at baseline (3 to 5 months after the CIS) and repeated after 12 months and every 5 years thereafter. Brain MRI was performed in two different 1.5 T magnet scans and included the following sequences: transverse proton density and T2-weighted conventional or fast spin-echo, transverse and sagittal T2-FLAIR, and unenhanced and contrast-enhanced (0.1–2.0mmol/kg; scan delay, 5–10 min) T1-weighted spin-echo (repetition time [TR] 500 ms, echo time [TE] 9 ms, flip angle=90, and voxel size 0.8 x 0.8 x 3 mm<sup>3</sup> for the first scan; and TR 725 ms, TE 14 ms, flip angle=70, and voxel size 0.9 x 0.9 x 5 mm<sup>3</sup> for the second scan). All sequences were obtained using a contiguous 3–5mm slice thickness covering the entire brain. Two neuroradiologists with experience in analyzing MR images in clinical studies and clinical trials related to MS, visually assessed the number and location of T2 lesions, and the fulfilment of Barkhof criteria [14],[15] at each time point. The number of Barkhof criteria was categorized as follows: 0 Barkhof criteria, 1 to 2 Barkhof criteria and 3 to 4 Barkhof criteria. The Statistical Parametric Mapping 8 (SPM8) software, a suite of MATLAB, was used to obtain global and regional (white and grey matter – WM and GM, respectively –) brain volumes 15 years after CIS. T1 lesion masks were created by a trained neurologist using JIM software; these masks were used to obtain a filled T1-weighted image that was used in the segmentation process to avoid misclassification

of focal white matter lesions as grey matter. Brain tissue masks obtained with Brain Extraction Tool (BET) [16] (part of the FSL library) were used to correct segmentation errors. Binary tissue brain masks (GM, WM, and cerebrospinal fluid – CSF –) were then visually inspected and patients with severe segmentation errors were excluded from the analysis. Total intracranial volume was calculated in order to normalize BVs and obtain an estimate of grey matter fraction (GMF), white matter fraction (WMF) and brain parenchymal fraction (BPF) for each patient.

*Statistical analysis.* We used the Statistical Package for the Social Sciences (SPSS) program (Chicago, Illinois) to analyse clinical and demographic data. All variables except for EDSS followed a normal distribution. Regression analyses were conducted to study the relationship between 15-year BVs and clinical and radiological outcomes. First, linear regression analyses were conducted including BPF, GMF, and WMF as dependent variables and age, gender, presence of oligoclonal bands (OB) in CSF, baseline EDSS, CIS topography, number of Barkhof criteria at baseline, presenting a second relapse during the follow-up, and a decision to start disease modifying treatment during the follow-up as independent variables. All significant variables were then included in three models of backward linear regression analysis, using again BPF, GMF, and WMF as dependent variables. Second, logistic regression analyses to predict reaching an EDSS of 3.0 during the 15-year follow-up period after CIS were performed using age, gender, presence of OB in CSF, baseline EDSS, CIS topography, number of Barkhof criteria at baseline, presenting a second relapse during the follow-up, a decision to start disease modifying treatment during the follow-up, and the different BV estimates as independent variables. All significant variables were then included in three models of backward multivariable logistic regression analysis together with BPF, GMF, and WMF in each model. As two different 1.5T MRI scans were used, when BVs estimates were being explored, all regression analyses were corrected by this

variable. Partial correlation coefficient ( $r_p$ ) for each independent variable in the regression models is reported.

## Results

Sixty-three patients were included in the study; four patients were excluded of the analysis due to movement artefacts and five patients due to segmentation errors, leading to a final cohort of 54 patients. Baseline clinical and demographical characteristics are detailed in table 1. There were no differences between included and excluded patients (data not shown). During the follow-up, 29 patients (53.7%) presented a second relapse after a median time of 28.7 months (range 3.1 – 164.6), and in 18 patients (33.3%) a decision to start a disease-modifying drug was made after a median time of 50.5 months (range 1.5 – 177.1).

*Brain volume estimates 15 years after a CIS.* Mean BPF, GMF and WMF at 15 years were 0.83 (SD 0.01), 0.44 (SD 0.01), and 0.39 (SD 0.02), respectively.

Results of the brain volume estimates by disease characteristics are detailed in table 2. In the linear regression analyses, lower values of BPF were significantly associated with the presence of OB in CSF ( $r_p=-0.475$ ,  $p=0.005$ ), presenting 3 to 4 Barkhof criteria at baseline ( $r_p=-0.403$ ,  $p=0.004$ ), presenting a second relapse ( $r_p=-0.380$ ,  $p=0.005$ ) during the follow-up, and with the decision to start treatment during the follow-up ( $r_p=-0.294$ ,  $p=0.036$ ). A trend towards a significant association between lower values of BPF and being male ( $r_p=0.251$ ,  $p=0.072$ ) was also found. Similarly, lower values of WMF were also associated with the presence of OB in CSF ( $r_p=-0.568$ ,  $p=0.001$ ), presenting 3 to 4 Barkhof criteria at baseline ( $r_p=-0.368$ ,  $p=0.009$ ), and presenting a second relapse ( $r_p=-0.406$ ,  $p=0.003$ ) during the follow-up. A trend towards a significant association between lower values of WMF and a decision to start treatment during the follow-up ( $r_p=-0.270$ ,  $p=0.055$ ) was also found. As for GMF, only age at CIS was associated with lower values of GMF ( $r_p=-0.413$ ,  $p=0.002$ ).

Results of the backward linear regression analyses including all significant variables are detailed in table 3. Lower values of BPF and WMF were associated with different inflammatory disease parameters whereas lower values of GMF were only associated with age at CIS.

*Predicting disability 15 years after a CIS.* Median EDSS score 15 years after CIS was 1.5 (range 0 – 6.5), and 10 patients (18.5%) reached an EDSS score of 3.0. In univariable logistic regression analyses, a higher baseline EDSS (OR 2.6, 95% CI 1.1 – 6.3,  $p=0.034$ ), presenting 3 to 4 Barkhof criteria (OR 10.1, 95% CI 1.1 – 102.8,  $p=0.039$ ), and a decision to start treatment during the follow-up (OR 28.4, 95% CI 3.1 – 258.4,  $p=0.003$ ) were significantly associated with a higher risk of reaching an EDSS of 3.0 during the follow-up. A trend towards a significant association between being male (OR 3.5, 95% CI 0.84 – 15.1,  $p=0.085$ ) and a higher risk of reaching an EDSS of 3.0 was also observed. Regarding BV estimates, in the univariate logistic regression analysis only a lower BPF was associated with a trend towards a higher risk of reaching an EDSS of 3.0 during the follow-up (OR 0.570, 95% CI 0.315 – 1.034,  $p=0.064$ ), and no association with GMF or WMF was observed (OR 0.635, 95% CI 0.322 – 1.250,  $p=0.189$ , and OR 0.849, 95% CI 0.537 – 1.341,  $p=0.482$ , respectively). Results of the backward logistic regression analyses including all significant variables together with BV estimates are detailed in table 4. A lower GMF at 15 years, but not BPF nor WMF, was associated with a higher risk of reaching an EDSS of 3.0 during the follow-up (OR 0.24, 95%CI 0.1-0.8,  $p=0.028$ ).

## Discussion

Our study reinforces previous results regarding GM changes being associated with disability [10],[12],[17], and adds to present knowledge of long-term BV loss and its relationship with clinico-radiological features in MS patients.

In this regard, in the multivariable analysis for predicting long-term BV loss, we have found that **lower** GM **values were** only associated with older age at CIS onset, whereas lower values of brain and WM volumes were related to male gender, presenting a higher number of Barkhof criteria at baseline MRI, developing a second relapse during the follow-up, and with the decision to start a disease modifying treatment. The fact that neither baseline nor follow-up disease variables could predict the development of GM volume loss 15 years after CIS reinforces the idea that GM pathology occurs independently from overt inflammation [18],[19] and is in accordance with previous literature [20],[21]. On the contrary, in this work we have found that global brain or WM measures were mostly related with inflammatory aspects of the disease, such as presenting a higher number of Barkhof criteria at baseline or presenting a second relapse during the follow-up, which is in line to what has been previously described [20]–[23]. Being male has been associated with a higher rate of BV loss, both in normal brain aging [24] as well as in MS patients [25], which is in concordance with the results provided in this work. Of note, we have also found that lower brain and WM volumes were associated with a decision to start treatment during the follow-up. The beneficial effect of disease modifying treatment on BV loss has been clearly demonstrated in both clinical trials and observational studies [26]. Owing to the observational design of the present study (lack of randomization and appropriate controls), the relationship between **lower** BV **values** and the onset of disease modifying therapies should be interpreted with caution. First, although treatment onset is included as a variable, whether the patient remained on the same treatment, needed a treatment change due to disease activity or whether treatment was withdrawn due to any other reason could not be included in

the analyses due to obvious limitations and complexities inherent to real-world studies. Of note, a decision to start treatment was made in only one third of the patients, and a bias to treat severe patients with a higher risk of poor outcomes it is most likely to have occurred. In fact, a significantly higher proportion of patients receiving treatment fulfilled 3 to 4 Barkhof criteria on the baseline MRI, and presented a second relapse during the follow-up as compared to patients that did not start treatment (data not shown).

In spite of only 18.5% of the sample reaching an EDSS of 3.0 during follow-up, we have found a significant association between lower GM volume and disability which is in line with previous work [10]–[12]. Moreover, the relationship between GM volume and disability remained significant (and in fact only appeared) when correcting for other significant variables, such as gender and baseline EDSS. In the multivariable analysis we have found that male gender confers a higher risk for disability accrual 15 years after CIS, which is still controversial in the literature [13],[28],[29]. The results of the multivariable logistic regression analysis may also mislead to conclude that starting a disease modifying treatment could be associated with a worse outcome, such as reaching an EDSS of 3.0. However, and as it has been previously mentioned, such results should be interpreted with caution as they may only reflect patients' disease severity.

Our work has some **methodological** limitations that should be noted. Ideally, when brain atrophy studies are performed, brain MRI should be acquired in the same scanner. However, and as it has been stated in the methods section, two different magnets were used for MRI acquisitions. In order to correct for this technical aspect, scan type was included as a covariate in all the statistical models used to predict 15-year BVs. Another limitation of our work is the cross-sectional BV **analysis as well as the lack of a control group**. Due to the long-term follow-up of this cohort it was impossible to retrieve baseline MRI scans in order to analyse longitudinal BV loss. Admittedly, such longitudinal analyses would have proven very difficult due to



inevitable system upgrades, implying sequence innovations and hardware improvements. In any case, the focus of the present work was not to establish whether BV loss occurs in MS patients or if it is greater than in healthy controls, but to study baseline and follow-up clinico-radiological correlations with BV measures 15 years after CIS presentation in a group of patients with a long disease duration and a homogeneous follow-up. Finally, two-dimensional T1 scans were used to obtain BV estimates instead of the recommended three-dimensional T1 scans [30]. However, the fact that we have been able to detect associations between BV estimates and disability measures despite not using the best scans for a BV analysis only reinforces our results. Lastly, it is worth mentioning that, in the multivariable logistic regression analysis, for most of the independent variables the OR had a wide confidence interval. This was most probable due to the small sample size. Therefore, although there is a clear association between these variables and the risk of reaching an EDSS of 3.0, the strength of the associations should be interpreted with caution.

In summary, global and WM volumes are associated with higher inflammatory activity at the time of the CIS and over the following years. Instead, the presence of greater inflammation does not seem to have an impact on GM volume, which is in fact the main determinant of clinical disability in MS.

## **Conflict of interests**

The study was partly supported by a Genzyme Foundation research grant

**Angela Vidal-Jordana** has received honoraria as speaker and/or for participation in Advisory Boards from Novartis, Roche, Sanofi-Genzyme, and Biogen.

**Jaume Sastre-Garriga** has received honoraria as speaker and for participation in Advisory Boards in the last 36 months from Novartis, Biogen, Merck, Ammirall, TEVA, Celgene and Genzyme.

**Deborah Pareto** has received honoraria as speaker from Novartis, and Genzyme.

**Carmen Tur** has received an ECTRIMS post-doctoral research fellowship in 2015. She has also received honoraria and support for travelling from Bayer-Schering, Teva, Merck-Serono and Serono Foundation, Biogen, Sanofi-Aventis, Novartis, and Ismar Healthcare

**Georgina Arrambide** has received compensation for consulting services from Biogen-Idec, research support from Novartis, and speaking honoraria from Sanofi-Aventis.

**Susana Otero-Romero** has received speaking honoraria from Serono Symposia and research support from Novartis.

**Elena Huerga** declares that there is no conflict of interest.

**Raquel Mitjana** declares that there is no conflict of interest.

**Cristina Auger** has received speaking honoraria from Novartis, Biogen and Stendhal.

**Mar Tintoré** has received received speaking honoraria and travel expenses for scientific meetings in the past with Ammirall, Bayer, Biogen Idec, Genzyme, Merck Serono, Novartis, Sanofi-Aventis, Roche and Teva.

**Alex Rovira** serves on scientific advisory boards for Biogen Idec, Novartis, Sanofi-Genzyme, and OLEA Medical, has received speaker honoraria from Bayer, Sanofi-Genzyme, Bracco, Merck-Serono, Teva Pharmaceutical Industries Ltd, Novartis and Biogen Idec, and has research agreements with Siemens AG and Icometrix.

**Xavier Montalban** has received speaking honoraria and travel expenses for participation in scientific meetings, has been a steering committee member of clinical trials or participated in advisory boards of clinical trials in the past years with Actelion, Almirall, Bayer, Biogen, Celgene, Genzyme, Merck, Novartis, Oryzon Genomics, Roche, Sanofi-Genzyme and Teva Pharmaceutical.

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