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Brain atrophy evolution and lesion load accrual in multiple sclerosis: a 2-year follow-up study

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Background To investigate in a large cohort of patients with multiple sclerosis (MS), lesion load and atrophy evolution, and the relationship between clinical and magnetic resonance imaging (MRI) correlates of disease progression.

Methods Two hundred and sixty-seven patients with MS were studied at baseline and two years later using the same MRI protocol. Abnormal white matter fraction, normal appearing white matter fraction, global white matter fraction, gray matter fraction and whole brain fraction, T2-hyperintense, and T1-hypointense lesions were measured at both time points.

Results The majority of patients were clinically stable, whereas MRI-derived brain tissue fractions were significantly different after 2 years. The correlation between MRI data at baseline and their variation during the follow-up showed that lower basal gray matter atrophy was significantly related with higher progression of gray matter atrophy during follow-up. The correlation between MRI parameters and disease duration showed that gray matter atrophy rate decreased with increasing disease duration, whereas the rate of white matter atrophy had a constant pattern. Lower basal gray matter atrophy was associated with increased probability of developing gray matter atrophy at follow-up, whereas gray matter atrophy progression over 2 years and new T2 lesion load were risk factors for whole brain atrophy progression.

Conclusions In MS, brain atrophy occurs even after a relatively short period of time and in patients with limited progression of disability. Short-term brain atrophy progression rates differ across tissue compartments, as gray matter atrophy results more pronounced than white matter atrophy and appears to be a early phenomenon in the MS-related disease progression. *Multiple Sclerosis* 2009; **15:** 204–211. http://msj.sagepub.com

Key words: atrophy; MRI; multiple sclerosis; T2 lesions

Introduction

The pathological features of multiple sclerosis (MS) include inflammation and demyelination, as well as neurodegeneration [1–12]. These heterogeneous features may, therefore, have a different impact on the patients' neurological traits. The lack of robust correlations between conventional magnetic resonance

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imaging (MRI) and clinical measures of disability is attributable to various shortcomings of both MRI and clinical assessment. Among them, there is the inability of conventional MRI to disentangle between reversible and irreversible MS pathological changes. Quantitative MRI studies have shown that the measurement of brain atrophy is a putative marker of the net effect of pathological processes leading to irreversible tissue damage, such as demyelination and axonal loss, and have suggested that the severity of atrophy may be a major contributor to the accrual of MS-related disability [13–18]. Several studies have addressed the issue of brain atrophy progression in the general MS population [19–23], as well as in selected relapsing-remitting (RR) cohorts [24–30], and clinically isolated syndromes [31,32]. Brain atrophy has been evaluated for time frames ranging from few months [27–29,33] to several years [20,21,23,24,30,32]. However, the time course of atrophy progression is still debated, as well as its relationship with other MRI and clinical markers of disease evolution.

In a previous multicenter study [13], we measured lesion load (LL), white matter (WM), gray matter (GM), and whole brain (wB) atrophy in a large population of patients with MS using a fully automated, operator-independent, multiparametric segmentation method [34,35], and we investigated the correlations between atrophy and LL, as well as those between clinical and MRI data. The present follow-up study was performed in a part of the same population and with identical methodologies, with the aim to investigate, in a large, unselected cohort of patients with MS, the evolution of brain LL and atrophy over a two-year follow-up period, and the relationship between putative clinical and MRI correlates of disease progression.

Patients and methods

Patients with established MS [36] were recruited from the outpatient clinics of six university hospitals (Second University of Naples, University "Federico II" of Naples, and Universities of Bari, Catanzaro, Catania, and Palermo) and a regional hospital (Potenza). The MRI machine, housed in a truck, traveled to the site locations to perform the same MRI protocol in all patients. All patients underwent a complete neurological examination on the same day as the MRI session. Clinical MS progression at follow-up was defined as a worsening of the Expanded Disability Status Scale (EDSS) score [37], depending on the baseline values (≥1 point increase if baseline EDSS was <5.5, ≥0.5 point increase if it was ≥5.5). The protocol was approved by local ethics committees and all participants gave written informed consent.

Image acquisition and analysis

The same scanner (1.0 T Genesis Signa; GE Medical Systems, Milwaukee, USA) and imaging protocol as in the cross-sectional study were used for follow-up MRI. Conventional spin echo sequences were acquired to obtain T1-weighted (TR/TE 600/15 msec, two averages) and dual echo (TR/TE 2300/15–90 msec, one average) images, with 90° flip angle and 256 × 192 matrix size. For each sequence, two interleaved sets of 16, 4-mm thick axial slices, covering the entire brain, were acquired. All the studies were segmented using a multispectral, fully-automated method, based on relaxometric characterization of brain tissues [34,35]. The program furnishes complete sets of multifeature images [R1(=1/T1), R2(=1/T2), proton density (N(H))-based] and segmented images of the following intracranial tissues: cerebrospinal fluid, GM, normal appearing WM (NAWM), abnormal WM (aWM: measure of LL as determined by the R1, R2, and N(H) information and morphological characteristics), global WM (gWM: the sum of NAWM and aWM), and wB (the sum of gWM and GM). To normalize for head size variability, the volumes of intracranial tissues were expressed as fractions (f) of intracranial volume (ICV, calculated for each subject as the sum of all intracranial tissues). An expert operator, blinded to patients' clinical conditions, counted the number of new T2-hyperintense and T1-hypointense lesions on follow-up scans by comparison with the baseline ones. To guarantee the correspondence between basal and follow-up segmented volumes, the following procedure was implemented in Matlab (The MathWorks, Natick, Massachusetts, USA). For each patient, binary maps of nonair voxels were obtained from both studies. A coregistration matrix was then obtained registering the follow-up study to the basal one [38,39] with Statistical Parametric Mapping 2 (http://www.fil.ion.ucl.ac.uk/spm). Using the above matrix, the follow-up map was resliced (nearest neighbor) into the basal space and multiplied by the basal map, obtaining the nonair map shared by both studies in the basal space. Reslicing this map by the inverse of the above coregistration matrix, we obtained the map of shared voxels also in the follow-up space. Both binary maps were multiplied by the corresponding segmented 3D data, and thus, tissue volumes shared by the two studies were calculated.

Quality control of data

All the MRI studies and the segmented images of voxels shared by both the studies were visually inspected by Diagnostic Imaging experts. Fifteen patients (4.1%) were not included in the subsequent

analysis because the suboptimal quality of one of the two studies caused coregistration or segmentation inaccuracies. Finally, the ICV, not variable over time in an adult population, was calculated for each pair of studies. The mean ICV variation (follow-up – basal acquisition) calculated for the patients included in the present study was 0.003%, with SE = 0.024%.

Statistical analysis

The Student's *t*-test was used for group comparisons. Bivariate correlations between baseline data and their variations during the follow-up period were assessed using the Pearson correlation coefficient. A stepwise polynomial regression procedure was used to assess the relationship between age corrected tissue fractions (GM-f and gWM-f and their changes over time) and disease duration (DD).

To account for normal age-related changes in MRI-measured brain volumes, we performed a linear regression analysis based on data obtained from 103 healthy controls (HC) in the cross-sectional study; the independent variable was age, whereas the dependent variables were individual MRI-derived tissue fractions. For each MRI parameter, we obtained an estimated normal value, adjustable for the subjects' age: NAWM-f = 35.952 + 0.086 × age; gWM-f = 35.961 + 0.086 × age; GM-f = 57.269 – 0.190 × age; wB-f = 93.229 – 0.104 × age. Based on this model, the expected two-year decreases of GM-f and wB-f in HC were –0.38 (i.e., –0.190 multiplied by 2, standard error: 0.000233) and –0.21 (i.e., –0.104 multiplied by 2, standard error: 0.000270); whereas gWM-f was expected to increase of 0.34 (i.e., 0.17 multiplied by 2, standard error: 0.000249). These values were used to calculate a cutoff to identify patients with MS whom GM-f and wB-f reduction were significantly different from HC (i.e., atrophy progression). The cutoff was not calculated for the WM because the linear regression analysis showed that gWM-f was expected to increase over time, consistently with previous data on healthy subjects [34,40].

The cutoff found for GM-f and wB-f was applied for a multivariate logistic regression model. This model was used to evaluate the effect of categorical and continuous variables on clinical and MRI markers of disease progression (i.e., EDSS worsening, tissue fraction decrease).

Results

We studied 267 of 597 patients enrolled in the cross-sectional study [13]. The mean interval between baseline and follow-up scans was 25.14 months (range: 24–26 months).

The loss of 55% of patients is a major limit in our study; nevertheless, the reasons for the patients loss were mainly related to the design of the study (multicenter, with a mobile MRI on a truck) rather than to the MRI protocol or to the postprocessing. Indeed patients were lost at follow-up for the following reasons: one of the centers participating in the cross-sectional study could not reallocate the MRI truck for the follow-up scanning session; all the MRI studies from one of the participating centers could not be used because of a major technical problem discovered at the time of the off-line postprocessing; some patients (approximately 6% of those from each center) could not be recalled within the two-week time frame when the MRI machine was made available at each local center; and finally, only 15 patients (4.1%) were not analyzed because of insufficient reliability of the coregistration or segmentation processes.

The study population consisted of 171 (64%) women and 96 (36%) men (women/men ratio = 1.8), aged from 16 to 66 years [mean age ± standard deviation (SD): 36.6 ± 9.6 years]. The clinical course was RR in 208 (77.9%) and Secondary Progressive in 59 (22.1%) patients. The mean age at disease onset was 27.4 years (range: 10–60, SD: 8.3 years), whereas the mean DD was 9.2 years (range: 1–38, SD: 8.29 years). Mean EDSS scores were 2.82 (range: 0.0–8.0, SD: 1.5) at baseline and 2.95 (range: 0.0–8.0, SD: 1.6) at follow-up. According to the established criteria on EDSS changes at follow-up, the clinical status significantly worsened in 52 patients (19.5%), was stable or improved in 160 patients (60%), and did not change significantly in the remaining 55 patients (20.5%). One or more clinical relapses occurred in 163 patients (61.0%) during the period between the baseline and the follow-up evaluations. In all, 172 patients (64.4%) were being treated with interferon, 25 (9.4%) with other immunomodulating therapies, whereas 70 (26.2%) were not receiving any disease-modifying therapy.

The cross-sectional intercorrelation between MRI parameters and between clinical and MRI data at follow-up is consistent with previous results [13]. In detail, we found a significant correlation ($P = 0.000$) between all segmentation values and EDSS (–0.423 with GM-f, –0.431 with wB-f, –0.256 with gWM-f, –0.220 with NAWM-f, and +0.267 with aWM-f). When comparing patients with stable or improved clinical status versus patients with progression of disability, we found that GM-f (47.56% vs 48.57%) and wBf (84.89% vs 86.45%) at baseline were significantly ($P < 0.05$) lower in patients with progression of disability.

Table 1 Magnetic resonance imaging segmentation characteristics of 267 patients; tissue volume at baseline and follow-up and tissue volume change (% of ICV)

	Mean value (SD)	Minimum	Maximum	*P* value
aWM-f – baseline	0.587 (0.727)	0.000	5.608	—
aWM-f – follow-up	0.655 (0.815)	0.003	5.356	<0.001
aWM-f change	+0.067 (0.222)	–1.110	+2.022	—
NAWM-f – baseline	37.17 (2.932)	26.993	43.368	—
NAWM-f – follow-up	36.98 (3.137)	25.811	43.701	<0.001
NAWM-f change	–0.186 (0.850)	–3.464	+2.249	—
gWM-f – baseline	37.758 (2.549)	30.545	43.481	—
gWM-f – follow-up	37.639 (2.654)	30.293	43.718	<0.001
gWM-f change	–0.119 (0.781)	–2.518	+2.235	—
GM-f – baseline	48.413 (3.337)	37.970	57.380	—
GM-f – follow-up	47.451 (3.174)	36.138	54.980	<0.001
GM-f change	–0.963 (0.898)	–4.203	+0.977	—
wB-f – baseline	86.171 (4.411)	69.9132	95.890	—
wB-f – follow-up	85.090 (4.411)	68.841	94.639	<0.001
wB-f change	–1.081(0.993)	–5.577	+1.324	—

Looking at conventional MRI data, we found an increase of T2 lesion number (mean: 3.8, SD: 3.8) and T1 lesion number (mean: 2.6, SD: 3.2) in 2 years.

Table 1 shows MRI-derived parameters at baseline and follow-up and their percentage of ICV changes in 2 years: a significant increase of aWM-f and significant decreases of NAWM-f, gWM-f, GM-f, and wB-f were observed. We then calculated the 2-year percent change from the baseline values for each tissue; we found an increase of +11.41% for aWM-f and a decrease of –0.50% for NAWM-f, –0.31% for gWM-f, –1.97% for GM-f, and –1.2% for wB-f.

The mean decrease of wB-f and GM-f in patients with MS (–1.081% and –0.963% of the ICV, respectively) was greater than what expected by linear regression analysis in HC (–0.38% and –0.21% of the ICV, respectively) over a 2-year period (*P* values: <0.001 and 0.002, respectively). With respect to the same expected control values, a significant greater decrease of wB-f and GM-f was observed, respectively, in 225 (84.3%) and 194 (72.7%) patients.

Table 2 presents the correlations between clinical and MRI data at baseline and variation of MRI-derived fractions during follow-up.

Even if the correlation coefficients were low, a higher basal aWM-f was related with a higher variation of aWM-f, NAWM-f, and gWM-f during follow-up. MRI measures at baseline were not related with EDSS change during the follow-up, whereas a higher basal GM-f was significantly related with a higher variation of GM-f during follow-up; in particular, this correlation is supported by the later statistical analysis (see below).

Figure 1 reports the best polynomial fittings of age corrected GM-f and gWM-f at baseline versus DD in years and shows that disease related GM loss was more rapid in the first years, while the rate of gWM atrophy had a constant pattern.

Figure 2 reports the best polynomial fitting of per year GM-f changes in the follow-up period, after correction for normal ageing GM-f loss. The resulting linear model was coherent with the GM-f fitting of Figure 1 showing a decreasing rate of GM atrophy over time. The corresponding figure for gWM-f is not reported because gWM-f changes did not show significant correlation with DD.

A multivariate logistic regression analysis was performed to determine those factors associated with clinical and MRI evidences of disease progression at follow-up (i.e., EDSS worsening, increasing

Table 2 Univariate correlations between clinical and magnetic resonance imaging parameters at baseline and variations during 2 years

Baseline values	Variations					
	EDSS	wB-f	GM-f	aWM-f	NAWM-f	gWM-f
EDSS	–0.133 (0.029)	—	—	—	–0.160 (0.009)	–0.148 (0.000)
wB-f	—	—	–0.179 (0.003)	–0.263 (0.000)	0.212 (0.000)	0.156 (0.000)
GM-f	—	—	–0.312 (0.000)	–0.180 (0.000)	0.244 (0.000)	0.215 (0.000)
aWM-f	—	—	—	0.269 (0.000)	–0.279 (0.000)	–0.227 (0.000)
NAWM-f	—	—	—	–0.251 (0.000)	—	—
gWM-f	—	—	—	–0.212 (0.000)	—	—

Values are Pearson correlation coefficients (two-tailed *P* values are in brackets).

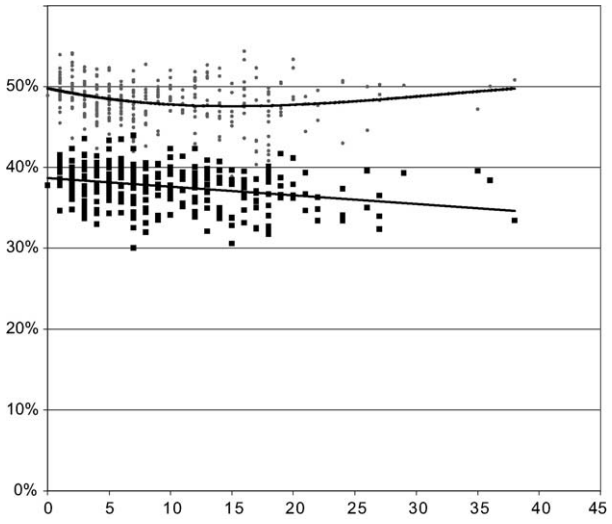

Figure 1 The best polynomial fitting of gray matter-f (GM-f) (gray dots, $P < 0.001$) and global white matter-f (gWM-f) (black squares, $P < 0.001$) at baseline versus disease duration (DD). Resulting equations are the following: 1) GM-f = 0.69×10^{-4} $DD^2 - 0.23 \times 10^{-2}$ DD + 0.49; 2) gWM-f = -0.11×10^{-2} DD + 0.39.

atrophy, and LL). A less severe GM atrophy (higher GM-f) at baseline was associated with increased probability of GM atrophy (lower GM-f) at follow-up [Odds ratio (OR) 1.14; 95% confidence interval [CI]: 1.03–1.26]. Furthermore, GM-f decreases over 2 years, but also new T2 lesions were associated with increased probability of wB atrophy at follow-up: OR and 95% CIs were 3.02 [1.34–6.42] and 4.49 [2.30–9.34]. Finally, a SP course of disease was a protective factor for GM atrophy evolution, OR and 95% CIs 0.34 [0.17–0.69].

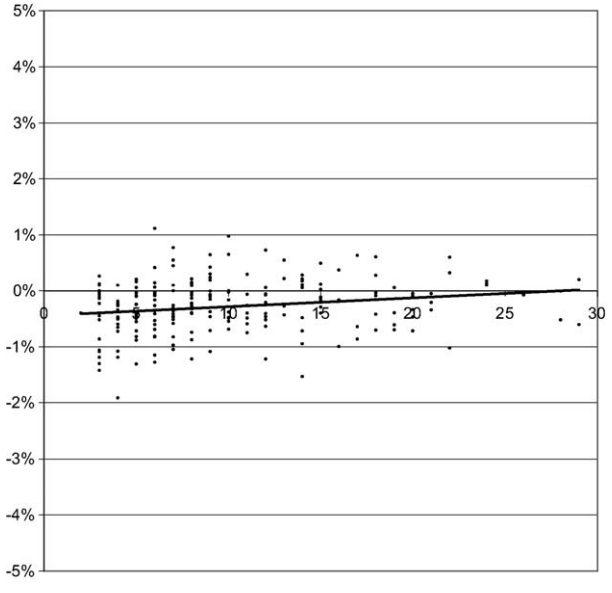

Figure 2 The best polynomial fitting (1° degree) of gray matter-f changes in the follow-up period versus disease duration in years in relapsing-remitting patients ($P < 0.005$).

Discussion

Numerous cross-sectional [15,41–43] and short-term longitudinal [26], MRI-based studies have shown that brain atrophy, with a predominant involvement of GM, occurs since the early clinical stages of MS, suggesting that the rate of atrophy progression is probably more relevant at the beginning of the disease, then reaches a cutoff above which the neurodegenerative process slows. Other longitudinal studies have investigated the course of LL and atrophy progression in MS, achieving conflicting results. In particular, long-term follow-up studies emphasized the possible correlation between early focal inflammatory WM pathology and subsequent brain atrophy development and clinical disease severity [20,30].

Among the pitfalls of these studies, there are either the limited sample sizes [23,26,32,33] or the use of heterogeneous acquisition schemes and the potential selection bias of patients participating in clinical trials [24,25,42]. Against this background, we performed this longitudinal study, which followed a previous, cross-sectional one [13], to investigate the dynamics of brain atrophy evolution and its relationship with clinical findings, in a large sample of unselected patients with MS who underwent the same scanning protocol.

Our findings indicate that even after a relatively short period of time, brain atrophy, with predominant involvement of GM, occurs in patients with MS, and it is not just an age-related phenomenon. On one hand, they are consistent with previous reports [15,18,25–28,31–33]. On the other hand, the sample we studied, which well reflects the real-life heterogeneity of MS, and the fully-automated method we used underpin the novelty of our study, which provides an important piece of evidence for the relevance of brain atrophy as a paraclinical marker of MS progression.

In detail, we found a significant decrease of NAWM-f, gWM-f, GM-f, and wB-f as well as a significant increase of T2 and T1 lesion number and aWM-f. Furthermore, when comparing the observed GM-f and wB-f decreases in patients with the corresponding predicted changes in HC, we found a significantly greater rate of GM-f and wB-f reduction in the former group.

A comparison between gWM-f value changes from patients with MS and controls was not done because in healthy subjects gWM-f was expected to increase over time, whereas in patients with MS it was decreased. Moreover, it should be taken into

account that the gWM-f includes both aWM-f and NAWM-f that may vary in different directions, with a possible compensatory effect on the cumulative tissue fraction of gWM. Nevertheless, gWM-f change in MS appears still ambiguous and difficult to interpret as the final result may depend on the interaction of a number of events, like inflammation, demyelination, axonal loss, and edema.

When comparing the rate of changes for the individual brain tissue compartments, it is worth noting that the highest percentage decrease was that of GM-f, which, after 2 years, was reduced on average by 1.97% of its baseline value. The magnitude of such reduction was three to four times higher than that observed for NAWM-f and gWM-f, which were approximately 0.5% and 0.3%.

Furthermore, the bivariate correlation analysis showed that a lower basal GM atrophy was significantly related with a higher progression of GM atrophy during follow-up. These findings were confirmed by the multivariate logistic regression analysis showing that a less severe GM atrophy (higher GM-f) at baseline was associated with increased GM loss (lower GM-f) at follow-up. Moreover, the polynomial regression showed that GM atrophy rate decreased with increasing DD, whereas WM atrophy had a constant decline over time. These data, in agreement with previous MRI and histopathological evidences [10,25,26,29,32], suggest that the rate of GM atrophy progression could be more relevant in the early stage of the disease, and then it may reach a threshold above which the neurodegenerative process would slow down, and that WM and GM pathology could proceed with mechanisms in part independent and having a different evolution over time.

Moreover, the multivariate analysis showed that having a progressive course of disease was a protective factor for GM atrophy progression. These data suggest that patients showing a SP course at baseline at this time had probably reached the cutoff above which the GM neurodegenerative process may then slow.

The underlying pathological substrates of the progressive GM loss observed in our patients might be the accumulation of GM lesions, often undetected when using conventional MRI, but which are known to harbor transected dendrites and axons, as well as the apoptotic neurons, thereby possibly contributing to a shrink of GM volume [44] in addition to the retrograde degeneration of neurons, following the injury to axons passing through diseased WM regions [1].

Moreover, as assessed by the multivariate analysis, the increase of T2 lesion number over 2 years was a risk factor to have wB atrophy at follow-up. These data suggest that even if focal WM pathology seems to be in part independent from GM pathology, and in general from the early diffuse tissue changes, it is however in part related to wB atrophy progression.

On the other hand, the correlation between atrophy and disability was assessed in two ways. The cross-sectional values at follow-up, in agreement with the literature and with our previously published work [13], showed a significant correlation between all segmentation fractions and EDSS, the stronger being the one with wB and GM atrophy.

Nevertheless, in the longitudinal observation, 80% of patients were free from significant clinical progression. The limited increase of EDSS we observed in our population at follow-up can be explained by the short duration of the follow-up period [45–48] and by the clinical profile of our patients, who were mainly RR and enrolled from outpatient clinics; moreover, we cannot rule out that patients who experienced a more severe progression could be part of those who did not participate in the follow-up.

When comparing the group of patients with progression of disability with patients with stable or improved clinical status, we found that GM and wB atrophy at baseline were significantly higher in patients with progression of disability.

Thus suggesting that these MRI parameters might be a potential predictor of clinical progression, although a longer follow-up may be needed for this pattern to become statistically significant.

Moreover, we did not find a relationship between changes of EDSS and those of MRI-derived measures during the study period. This may be largely due to the paucity of the observed changes of EDSS scores discussed before. Another potential reason is the limited reliability and responsiveness of the EDSS scale, which is also insensitive to cognitive impairment and might have failed to detect the possible worsening of patients' neuropsychological performances during the study period, if any. Furthermore, the progression of GM atrophy might have been accompanied by cortical reorganization, which is known to limit the impact of brain injury on the severity of neurological impairment in MS [49–52]. The use of MRI methodologies able to assess intrinsic (i.e., diffusion tensor MRI or proton MR spectroscopy) or regional (i.e., cortical thickness measurement or based morphometry) tissue damage may also enable us to strengthen the poor relationship we observed between the progression of GM atrophy and that of MS-related disability; furthermore, the assessment of cord damage may complement that of brain injury to generate multiparametric MR-based scores and achieve an optimal paraclinical assessment of MS severity. In any case, our data suggest that GM atrophy evolution might be related with MS clinical progression, although, as reported in a recently

published long term follow-up work [32], longer periods of observation are needed to improve the comprehension of longitudinal clinical-MRI correlation.

Finally, one cannot dismiss that the MRI acquisition protocol and the postprocessing analysis used in the present study are relatively easy and fully automated, thus suggesting that after validation by a longer follow-up, they may represent a possible tool to implement the management of individual patients.

Disclosure

The authors report no conflicts of interest.

The statistical analysis was performed by Orietta Picconi, DStat., "Agenzia di Sanità Pubblica Regione Lazio."

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