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ORIGINAL ARTICLE

Cerebellar pathology and disability worsening in relapsing-remitting multiple sclerosis: A retrospective analysis from the CombiRx trial

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Abstract

Background and purpose: Cerebellar damage is a valuable predictor of disability, particularly in progressive multiple sclerosis. It is not clear if it could be an equally useful predictor of motor disability worsening in the relapsing-remitting phenotype.

Aim: We aimed to determine whether cerebellar damage is an equally useful predictor of motor disability worsening in the relapsing-remitting phenotype.

Methods: Cerebellar lesion loads and volumes were estimated using baseline magnetic resonance imaging from the CombiRx trial ($n = 838$). The relationship between cerebellar damage and time to disability worsening (confirmed disability progression [CDP], timed 25-foot walk test [T25FWT] score worsening, nine-hole peg test [9HPT] score worsening) was tested in stagewise and stepwise Cox proportional hazards models, accounting for demographics and supratentorial damage.

Results: Shorter time to 9HPT score worsening was associated with higher baseline Expanded Disability Status Scale (EDSS) score (hazard ratio [HR] 1.408, $p = 0.0042$) and higher volume of supratentorial and cerebellar T2 lesions (HR 1.005 $p = 0.0196$ and HR 2.211, $p = 0.0002$, respectively). Shorter time to T25FWT score worsening was associated with higher baseline EDSS (HR 1.232, $p = 0.0006$). Shorter time to CDP was

associated with older age (HR 1.026, $p = 0.0010$), lower baseline EDSS score (HR 0.428, $p < 0.0001$) and higher volume of supratentorial T2 lesions (HR 1.024, $p < 0.0001$).

Conclusion: Among the explored outcomes, single time-point evaluation of cerebellar damage only allows the prediction of manual dexterity worsening. In clinical studies the selection of imaging biomarkers should be informed by the outcome of interest.

INTRODUCTION

Cerebellar atrophy is relevant to clinical disability in multiple sclerosis (MS) [1]. In progressive MS, atrophy of specific cerebellar lobules explains different aspects of motor and cognitive disability [2,3] and cerebellar atrophy is a significant predictor of sustained disability [4]. In the relapsing-remitting (RR) phenotype, a cross-sectional analysis has identified a relationship between cerebellar volume, fatigue and depression [5]. Moreover, several studies, conducted in mixed samples including RRMS patients, suggest a relationship between cerebellar atrophy and motor and cognitive disability [6–12], with a recent study showing a significant role for baseline cerebellar gray matter (GM) volume as predictor of 1-year Expanded Disability Status Scale (EDSS) score worsening [13]. Although cerebellar atrophy has been suggested as a potential short-term imaging metric to monitor treatment effect in primary-progressive MS [4], it is not clear if cerebellar volume could be an equally useful predictor of motor disability worsening, as commonly assessed in clinical trials, in RRMS.

As per the role of cerebellar lesions, both cortical and white matter (WM) lesions correlate with clinical disability in RRMS [14–16], however, brainstem and spinal cord lesions, rather than cerebellar lesions, seem to significantly predict EDSS score worsening over time in relapsing-onset MS[17] and clinically isolated syndrome [18].

Given this background, we performed a retrospective analysis in a large cohort of RRMS patients prospectively enrolled in the CombiRx clinical trial[19] to better define the role of cerebellar damage (lesions and volume) as a meaningful biomarker of motor disability worsening in RRMS, while accounting for the effect of demographic variables and supratentorial damage.

METHODS

Study design

We conducted a retrospective analysis of magnetic resonance imaging (MRI) and clinical data from the CombiRx trial (NCT00211887). CombiRx was a 3-year, double-blind, multicenter randomized clinical trial that tested the efficacy of a combined therapy of interferon-β1a plus glatiramer acetate versus the efficacy of either agent alone, and provided for double-blind therapy until the last participant completed the 3-year trial, allowing up to 7 years of observation of some participants.

Inclusion criteria for the trial were: confirmed diagnosis of RRMS (by Poser or McDonald criteria) with two or more exacerbations in the prior 3 years; age between 18 and 60 years; EDSS score of 0 to 6; and relapse-free status for at least 30 days preceding screening and randomization. Patients who completed the 3-year placebo-controlled core study were eligible for inclusion in the extension phase, with up to 7 years of total follow-up. As per trial protocol, participants were followed every 12 weeks with neurological assessments. EDSS examinations occurred every 12 weeks in the core study through month 42 and every 6 months thereafter in the extension. The Multiple Sclerosis Functional Composite assessment was performed quarterly in the core study and annually in the extension study. MRI was performed at study entry, months 6, 12, 24, 36, 48 and 60, and at the end of study visit in the extension.

In the present study, we investigated the role of baseline supratentorial and cerebellar pathology as predictors of time to disability worsening over the CombiRx study period (core study + extension phase).

The trial protocol and its amendments received approval from the applicable central or institutional review boards and the National Institutes of Health (NIH)-appointed study data and safety monitoring committee before site initiation and recruitment of participants. Informed consent was obtained prior to any screening procedures or enrollment.

Clinical outcomes of interest

Disability worsening was assessed in terms of: (i) time to confirmed disability progression (CDP), defined as a 1.0 increase in the EDSS score from baseline, when baseline score was ≤5.0; or an increase of 0.5 from baseline, when baseline score was ≥5.5, sustained for 6 months (two successive quarterly visits); (ii) time to first 20% change in timed 25-foot walk test (T25FWT) score; and (iii) time to first 20% change nine-hole peg test (9HPT) score.

MRI acquisition and analysis

The MRI protocol included two-dimensional fluid attenuated inversion recovery, dual-echo turbo-spin echo, and isotropic pre- and

post-contrast T1-weighted images (0.94 mm × 0.94 mm × 3 mm voxel dimension). The three-dimensional T1-weighted images were acquired either with spoiled gradient recalled echo (SPGR) or magnetization prepared rapid gradient echo (MPRAGE) sequences (0.94 mm × 0.94 mm × 1.5 mm voxel dimension).

Baseline MRI datasets were screened to assess their suitability for volumetric analysis. Of the original 1008 datasets available, 109 were excluded because of incomplete cerebellar coverage or presence of cerebellar artifacts. In the remaining 899 datasets, supratentorial and cerebellar T2 hyperintense and gadolinium-enhancing (Gd+) lesion number and volumes, together with GM volumes, were computed as follows. Cerebellar T2-weighted hyperintense lesions and T1-weighted Gd+ lesions were segmented by one experienced observer using a semiautomatic segmentation technique (Jim 7; Xinapse Systems, West Berghol, UK), deriving both cerebellar lesion volume and number. Supratentorial T2-weighted hyperintense lesions and T1-weighted Gd+ lesion volume and number were computed as the difference between whole-brain lesion volume/number estimated during the trial and cerebellar lesion volume/number.

Cerebellar volumes (cerebellar GM, anterior cerebellum volume, posterior cerebellum volume) were calculated on lesion-filled T1-weighted images using the Spatially Unbiased Infratentorial Toolbox (SUIT) version 3.2, implemented in Statistical Parametric Mapping (SPM)12, as described in Cocozza et al. [2]. During this step, 61 datasets were excluded because of cerebellar segmentation failure. Briefly, cerebellar segmentation output was visually assessed by an experienced observer (M.P.) in order to ascertain that all cerebellar lobules were included in the final mask and that no extracerebellar structures (transverse and sigmoid sinuses, brainstem) were erroneously classified as cerebellar tissue. If any of the abovementioned tissue classification errors was noted, the participant was excluded from further analyses. The remaining 838 datasets, constituting the final study population, were further processed to obtain supratentorial brain volumes. Briefly, lesion-filled T1-weighted images were segmented into GM, WM and cerebrospinal fluid using the segmentation tool implemented in SPM12 software (http://www.fil.ion.ucl.ac.uk/spm). For each participant, intracranial volume (ICV) was computed as the sum of GM, WM and cerebrospinal fluid. Supratentorial GM volume was then computed as the difference between whole-brain GM volume and cerebellar GM volume.

Statistical analysis

Descriptive analyses (means, medians, standard deviations, interquartile ranges, frequency distributions) were performed to assess and describe the study population. As supratentorial and cerebellar volumes both partly depend on individual biological features (ICV), and both are affected by MS pathogenic processes, bivariate correlations between ICV, supratentorial volume and cerebellar volume were tested. Stagewise Cox proportional hazards models were built to assess the effect of baseline MRI features on time to CDP, and time to 20% T25FWT and 9HPT score worsening, assessing the models at

TABLE 1 Demographic and clinical features of the study population (N = 838)

Age, years	37.70 ± 9.66
Women, n (%)	607 (72.4)
Disease duration from symptom onset, years	
Mean ± SD	4.22 ± 5.53
Median (IQR)	2 (1–5)
Disease duration from diagnosis, years	
Mean ± SD	1.07 ± 3.11
Median (IQR	0 (0–1)
Median (range) EDSS score	2 (0–6)
T25FWT score	4.99 ± 1.70
9HPT score	20.17 ± 4.81

Abbreviations: 9HPT, nine-hole peg test; EDSS, Expanded Disability Status Scale; IQR, interquartile range; SD, standard deviation; T25FWT, timed 25-foot walk test.

each stage. In stage I, demographic and clinical features (age, gender, baseline EDSS score) were entered, in stage II, supratentorial MRI features (T2 hyperintense lesion volume and number, Gd+ lesion volume and number, supratentorial GM volume) were added and, in stage III, cerebellar MRI features (T2 hyperintense lesion volume and number, Gd+ lesion volume and number, cerebellar GM volume [with total volume as predictor in the models predicting CDP and anterior cerebellar volume as predictor in the models predicting time to T25FWT/time to 9HPT score worsening]) were added. After each of the three stages, a stepwise forward selection model was used to select variables until no more explanatory power was available for the model. The statistical analysis was conducted in SAS (version 9.4), with p values <0.05 considered as statistically significant and with no adjustments for the multiplicity of testing as we considered this a discovery cohort.

RESULTS

Study population

The demographic, clinical and MRI characteristics of the study population are shown in Tables 1 and 2.

Relationship between supratentorial and infratentorial volumes

As expected, supratentorial and cerebellar volumes were correlated. Specifically, bivariate correlations between ICV, supratentorial volume and cerebellar volume are as follows: ICV–supratentorial volume (r = 0.628, p < 0.01); ICV–cerebellar volume (r = 0.606, p < 0.01); supratentorial volume–cerebellar volume (r = 0.575, p < 0.01).

TABLE 2 MRI features of the study population (N = 838)

Supratentorial Gd+ lesions number	1.74 ± 4.30
Supratentorial Gd+ lesions volume, ml	0.11 ± 0.35
Supratentorial T2 lesions number	86.49 ± 55.49
Supratentorial T2 lesions volume, ml	10.52 ± 11.59
Cerebellar Gd+ lesions number	0.03 ± 0.24
Cerebellar Gd+ lesions volume, ml	0.004 ± 0.03
Cerebellar T2 lesions number	0.92 ± 1.82
Cerebellar T2 lesions volume, ml	0.12 ± 0.30
Supratentorial GM volume, ml	562.33 ± 73.48
Cerebellar GM volume, ml	109.78 ± 11.64
Anterior cerebellum volume, ml	14.35 ± 1.62
Posterior cerebellum volume, ml	95.43 ± 10.20

Note: All values are reported as mean ± standard deviation.

Abbreviations: Gd+, gadolinium enhancing; GM, gray matter.

Time to 9HPT worsening

The first stage of the stagewise model identified significant associations between time to 9HPT worsening, female gender and baseline EDSS score ($p = 0.0435$ and $p < 0.0001$, respectively). At the second stage, entering supratentorial MRI variables, baseline EDSS score remained significantly associated with time to 9HPT worsening ($p = 0.0028$), together with age ($p = 0.0059$), volume of supratentorial Gd+ and T2 lesions ($p = 0.0260$ and $p = 0.0061$, respectively), and supratentorial GM volume ($p = 0.0401$). At the third stage, when entering cerebellar MRI variables, anterior cerebellar volume was significantly associated with time to 9HPT worsening ($p = 0.0444$). The stepwise selection confirmed the association between shorter time to 9HPT worsening, higher baseline EDSS score (hazard ratio [HR] 1.408, $p = 0.0042$) and higher volume of supratentorial T2 lesions (HR 1.005, $p = 0.0196$), and highlighted an association between higher volume of cerebellar T2 lesions and shorter time to 9HPT score worsening (HR 2.211, $p = 0.0002$).

Time to T25FWT worsening

The stagewise model identified significant associations between time to T25FWT worsening and baseline EDSS score ($p = 0.0032$). The stepwise selection confirmed the association between shorter time to T25FWT worsening and higher baseline EDSS score (HR 1.232, $p = 0.0006$).

Time to confirmed disability progression

At the final stage of the stagewise modeling, significant associations between time to CDP, age, and baseline EDSS score were found

($p = 0.0009$ and $p < 0.0001$, respectively). Among the supratentorial variables, volume of supratentorial T2 lesions was associated with CDP ($p = 0.0020$), but the association was lost once number and volume of cerebellar Gd+ lesions were entered into the model ($p = 0.0389$ and $p = 0.0223$, respectively). The stepwise selection confirmed the association between shorter time to CDP, older age (HR 1.026, $p = 0.0010$), lower baseline EDSS score (HR 0.428, $p < 0.0001$) and higher volume of supratentorial T2 lesions (HR 1.024, $p < 0.0001$).

DISCUSSION

Through our retrospective analysis, we set out to explore the relevance of cerebellar lesions and volumes as predictors of different outcomes of motor disability worsening in RRMS. Our results suggest that cerebellar damage, evaluated at a single time point, is a useful predictor of worsening only in selected functional domains (i.e., manual dexterity). Indeed, in our population, supratentorial and cerebellar lesion load were associated with shorter time to 9HPT score worsening, confirming previous findings in patients with progressive MS, where WM damage (brain and cerebellar lesion volumes/WM microstructural damage), significantly predicted 9HPT score in cross-sectional analyses [2,7]. Although atrophy of salient cortical and cerebellar regions might contribute to manual dexterity impairment [6,10], focal lesions disrupting WM tracts involved in hand motor control most likely represent the main pathophysiological substrate of 9HPT score worsening [11,20]. Among clinico-demographic features, baseline EDSS score significantly predicted 9HPT score worsening, in agreement with a recent longitudinal assessment of hand function in patients with MS [21].

Regarding the risk of T25FWT score worsening, this was greater in patients with higher baseline EDSS scores. This is in line with studies investigating the natural history of clinical outcomes in progressive MS [22,23], and can be explained considering the nonlinear relationship between T25FWT and EDSS scores, with the times to T25FWT score worsening increasing exponentially once a threshold of EDSS 5.5 is reached [23]. As for the role of brain and cerebellar MRI metrics, no significant predictor was identified, which is similar to findings in patients with progressive MS and those with relapsing-onset MS [2,6]. In fact, ambulation worsening and the need for a walking aid are known to be preferentially associated with damage of other districts, such as deep GM and spinal cord [24–26].

Finally, a shorter time to CDP was identified in older patients, which is not surprising given that aging is associated with a reduced structural central nervous system reserve, a delayed and incomplete recovery from relapses and a decline in disease-modifying therapy efficacy [27]. Although patients with lower disability at baseline seemed more likely to reach CDP faster, this finding must be interpreted with caution, as patients at the lower EDSS levels are exposed to more measurement error which, once corrected at subsequent evaluations, would determine an apparent progression not associated with an actual change in their clinical status. Additionally,

a plateau effect is usually seen with EDSS scores >4, which might favor the detection of CDP in the lower EDSS score group. From an MRI perspective, higher lesion load in the supratentorial compartment was associated with shorter time to CDP. Although atrophy is the most widely applied MRI marker of neurodegeneration and predictor of irreversible disability in MS [13,28–30], supratentorial WM lesion density and accrual significantly account for disability and short-term progression in RRMS [31], with WM lesion load being a relevant contributor in machine-learning models predicting disability over a medium-term follow-up [32]. Our finding, although based on a cross-sectional analysis, seems in line with recent work that, classifying distinct MS subtypes based on pathology-derived MRI features, has identified the lesion-led phenotype, characterized by early accrual of lesions, as the subtype with the highest risk of CDP [33]. As for the role of the cerebellum, although in the stage-wise model cerebellar lesions did show a significant association with time to CDP, the stepwise selection did not confirm their relevance. Again, this is in line with observations in patients with progressive MS, where changes in cerebellar volume over time, rather than baseline cerebellar metrics, were relevant for prediction of CDP at 1 year [4]. Indeed, the relatively modest contribution of cerebellar features at baseline to the predictive power of our models does not mean this can be assumed to be the case for longitudinal MRI measures.

Overall, baseline clinical features and lesion loads emerge as the most meaningful predictors in our models. Lesion loads, although generally showing poorer correlations with clinical status than atrophy, are less subject to the heterogeneity of the MRI scanners and/or acquisition protocols influencing consistency in brain atrophy measurements [34], which might partially account for the poor performance of volumetric measures in our analysis.

The present study has some limitations. First, the technical requirements for cerebellar segmentation prevented the inclusion of the full trial population, reducing the power of our analysis. Second, we choose to derive our imaging biomarkers from the baseline MRI acquisition, rather than analyzing MRI changes over time, which have shown a stronger predictive power than cross-sectional assessments [4]. Again, this choice is related to the fact that screening and exclusions of inadequate images from the follow-up examinations would have further reduced our sample and therefore the power of our investigation.

In conclusion, our findings, beyond confirming the role of cerebellar lesions as predictors of manual dexterity worsening over time, further underline the importance, when designing clinical trials, of accurate selection of the candidate imaging biomarkers according to the outcome of interest.

CONFLICT OF INTERESTS

AUTHOR CONTRIBUTIONS

Maria Petracca: Conceptualization (supporting); Data curation (lead); Writing – original draft (lead); Writing – review and editing (equal). **Gary Cutter:** Conceptualization (equal); Data curation (equal); Formal analysis (lead); Investigation (equal); Methodology (equal); Software (equal); Writing – review and editing (equal). **Sirio Cocozza:** Formal analysis (equal); Writing – review and editing (equal). **Leorah Freeman:** Investigation (equal); Writing – review and editing (equal). **John Kangarlu:** Data curation (equal); Formal analysis (supporting); Writing-review & editing (equal).

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DATA AVAILABILITY STATEMENT

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