Spinal cord atrophy in a primary progressive multiple sclerosis trial: Improved sample size using GBSI

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\textbf{ABSTRACT}

\textbf{Background:} We aimed to evaluate the implications for clinical trial design of the generalised boundary-shift integral (GBSI) for spinal cord atrophy measurement.

\textbf{Methods:} We included 220 primary-progressive multiple sclerosis patients from a phase 2 clinical trial, with baseline and week-48 3DT1-weighted MRI of the brain and spinal cord (1 × 1 × 1 mm\textsuperscript{3}), acquired separately. We obtained segmentation-based cross-sectional spinal cord area (CSA) at C1-2 (from both brain and spinal cord MRI) and C2-5 levels (from spinal cord MRI) using DeepSeg, and, then, we computed corresponding GBSI.

\textbf{Results:} Depending on the spinal cord segment, we included 67.4–98.1% patients for CSA measurements, and 66.9–84.2% for GBSI. Spinal cord atrophy measurements obtained with GBSI had lower measurement variability than corresponding CSA. Looking at the image noise floor, the lowest median standard deviation of the MRI signal within the cerebrospinal fluid surrounding the spinal cord was found on brain MRI at the C1-2 level. Spinal cord atrophy derived from brain MRI was related to the corresponding measures from dedicated spinal cord MRI, more strongly for GBSI than CSA. Spinal cord atrophy measurements using GBSI, but not CSA, were associated with upper and lower limb motor progression.

\textbf{Discussion:} Notwithstanding the reduced measurement variability, the clinical correlates, and the possibility of using brain acquisitions, spinal cord atrophy using GBSI should remain a secondary outcome measure in MS studies, until further advancements increase the quality of acquisition and reliability of processing.

1. Introduction

Spinal cord atrophy is a common feature of multiple sclerosis (MS), can be detected in vivo using MRI, and is one of the main substrates of disease progression (Furby et al., 2010; Rashid et al., 2006; Gass et al., 2015; Rocca et al., 2011). As such, spinal cord atrophy can be used to monitor disease progression, and has been included in clinical trials evaluating medications with putative neuroprotective effects in MS (Brownlee et al., 2017; Kearney et al., 2015, 2014; Dawley et al., 2018).

In our previous studies (Moccia et al., 2019; Prados et al., 2020), we have adapted the boundary-shift integral (BSI) technique developed for the brain, to be applied to the spinal cord, obtaining the first registration-based method for longitudinal assessment of spinal cord atrophy. This technique, called generalised BSI (GBSI) (Moccia et al., 2019; Prados et al., 2020), significantly reduced measurement error by providing a direct comparison in a joint analysis (i.e., registration of all images in a common space) (Moccia et al., 2019; Altmann et al., 2009), when compared with an indirect comparison of separate segmentations (i.e., numerical subtraction of segmentation values at different time-points) (Moccia et al., 2019). The latter approach can be compromised by differences in spinal cord coverage, miss-segmentations and/or changes in spinal cord curvature (Prados and Barkhof, 2018; Moccia et al., 2019). Reductions in measurement noise using GBSI could be particularly relevant to clinical trials, that, so far, have failed to show...
any significant treatment effect on spinal cord atrophy, especially in progressive MS patients (Giovannoni et al., 2020; Ciccarelli et al., 2019), where this outcome measure provides the strongest clinical correlates (Moccia et al., 2019; Ciccarelli et al., 2019).

The previous findings on the use of GBSI need to be confirmed in larger cohorts of progressive MS, including a broader range of clinical and patient-reported outcomes. Additionally, registration-based spinal cord atrophy measurements should be compared at different spinal cord levels and, ideally could be derived also from brain scans. In the present study, we re-analysed a phase 2 clinical trial on primary progressive MS (PPMS) to: 1) compare spinal cord atrophy measurements using segmentation- and registration-based methods, with possible implications for clinical trial design (e.g., measurement variability, image noise floor); 2) compare spinal cord atrophy measurements obtained from routine brain (C1-2) and dedicated spinal cord MRI (C1-2 and C2-5), using segmentation- and registration-based methods; 3) explore possible clinical correlates, also in relation to conventional brain MRI measures; and 4) explore possible treatment effect.

2. Methods

2.1. Study design

This is a secondary analysis on PPMS patients who participated in the ARPEGGIO phase 2 clinical trial (A Randomized Placebo-controlled trial Evaluating Laquinimod in primary progressive multiple sclerosis, Gauging Gradations In MRI and Clinical Outcomes). The ARPEGGIO trial was a randomised, double-blind, parallel-group, placebo-controlled study. Patients were randomised in a 1:1:1 ratio to receive oral Laquinimod 0.6 mg or 1.5 mg, or placebo once daily, from January 2015 to April 2016, at 85 sites in 10 countries. Duration of the core study was 48 weeks (Giovannoni et al., 2020).

2.2. Population

Inclusion and exclusion criteria have been reported previously (Giovannoni et al., 2020). Briefly, inclusion criteria were: aged 25–55 years; diagnosis of PPMS (Polman et al., 2011); 3.0–6.5 on the Expanded Disability Status Scale (EDSS) at baseline; documented worsening of clinical disability in the 2 years prior to screening; and a Functional System Score (FSS) ≥ 2 for the pyramidal system or gait impairment due to lower limb dysfunction. Exclusion criteria were: clinical history of any MS exacerbations or relapses; any other neurological disorder (e.g., cervical spinal cord compression, vitamin B12 deficiency); previous use of immunosuppressive/cytotoxic agents, experimental/investigational drugs and/or MS-specific treatments (e.g., Fingolimod, Dimethyl Fumarate, Glatiramer Acetate, Interferon-β, Laquinimod).

2.3. MRI acquisition and processing

Sagittal 3D T1-weighted isotropic images (1 × 1 × 1 mm^3) of the brain and the cervical spinal cord were acquired separately at baseline, week 24 and week 48, within 14 days of the scheduled clinical visit. MRI scans were acquired using scanners from multiple vendors with different field strengths (e.g., 1.5 T and 3 T), and using different acquisition parameters (optimised for each), following site certification; no software/hardware upgrades were allowed between baseline and follow-up scans. MRI scans were quality controlled and collected centrally at the VUmc in Amsterdam. For the purposes of the present study, we included the baseline and week-48 MRI scans.

For the primary analysis of the ARPEGGIO trial, spinal cord area at C1-2 level was determined using the NeuroQLab, a segmentation-based method using a Gaussian mixture modelling (Lakas et al., 2004; Liu et al., 2016; Daams et al., 2014). After definition of the spinal cord subsegment to be segmented, watershed segmentation of the spinal cavity and surrounding cerebrospinal fluid was performed, and mean upper cervical spinal cord area (MUCCA) was computed (Daams et al., 2014). Percent change of spinal cord area was calculated using the following formula: MUCCA = 100*(week 48 MUCCA - baseline MUCCA)/baseline MUCCA.

In the present study, masks of C1-2 and C2-5 levels were obtained from dedicated spinal cord MRI acquired at each time point, using the DeepSeg tool within the Spinal Cord Toolbox (version 4.0), a fully-automated segmentation method based on convolutional neural networks, which automatically identified different spinal cord levels (Fig. 1) (De Leener et al., 2017; McCoy et al., 2019). Similarly, masks of upper spinal cord (C1-2 level) were obtained from brain MRI, when this part of the anatomy was included in the sagittal acquisition (Fig. 2). Percent change of cross-sectional spinal cord area (CSA) was calculated using the following formula: CSA = 100*(week 48 CSA - baseline CSA)/baseline CSA. For GBSI, we followed the previously described pipeline (Moccia et al., 2019; Prados et al., 2020). Briefly, after straightening the spinal cord at both time points, a 3D symmetric and inverse-consistent affine registration to the half-way space between baseline and follow-up images was performed using 9 degrees-of-freedom (DOF) (translation, rotation and skew, each in three directions); masks were resampled to the same space using linear interpolation to the halfway space. This method does not generate any bias between baseline and follow-up images as the exact same image processing pipeline is applied to both timepoints. The probabilistic boundary-shift region-of-interest was then adaptively estimated from baseline and follow-up spinal cord segmentations. The GBSI was ultimately computed and the percent volume change was measured (Fig. 1; Fig. 2). For subsequent analyses, we excluded scans that showed ± 5% atrophy rate for both CSA and GBSI measurements at a given spinal cord level (e.g., ± 5% atrophy rate on both CSA at C1-2 level from brain MRI and GBSI at C1-2 level from brain MRI, on both CSA at C1-2 level from spinal cord MRI and GBSI at...
C1-2 level from spinal cord MRI, or on both CSA at C2-5 level from spinal cord MRI and GBSI at C2-5 level from spinal cord MRI). The 5% cut-off corresponds to 2 standard deviations in the rate of annual spinal cord loss in healthy controls in our previous study (Prados et al., 2020).

Looking at brain MRI, from the original clinical trial dataset, we extracted number of new T2 lesions, T2 lesion volume change, T1 lesion volume change, and percent brain volume change (PBVC), at baseline and week 48 visits. Full details of acquisitions and processing have been previously reported (Giovannoni et al., 2020).

2.4. MRI noise floor

To classify scans based on the noise floor, we used the $\sigma$ (standard deviation of the MRI signal), calculated with the following formula:

$$\sigma = \frac{\sigma_0}{\sqrt{2}}$$

where $\sigma_0$ is the standard deviation of the magnitude-$r$-reconstructed cerebrospinal fluid (CSF) signal (Jones and Basser, 2004; Tabelow et al., 2015; Battiston et al., 2018). In particular, the CSF ring surrounding the spinal cord was derived by dilation of the spinal cord mask by 2-pixel layer, and, then, by subsequent subtraction of the mask once; any value of voxels within the extracted ring that were $>2$ standard deviations above the mean were discarded to avoid the inclusion of values from nerve roots or other spurious signal intensities (Prados et al., 2020; Zhou et al., 2018; Sakaie et al., 2018). The standard deviation of the MRI signal in this CSF ring was calculated to compute the root power of the noise, and, then, used to account for the presence of noise floor. As such, $\sigma$ provides the scaled signal intensity of the images accounting for the presence of noise floor (rectified noise floor), independently from the magnitude of the CSF signal (Jones and Basser, 2004). This is a commonly applied measure for T1-weighted images, where signal from the CSF is suppressed (expected to be close to 0) (Jones and Basser, 2004+), and was automatically computed during GBSI pipeline, for each spinal cord level (Prados et al., 2020). For the purpose of our study, we classified scans based on the median $\sigma$ for each spinal cord segment.

2.5. Clinical variables

Baseline clinical variables were age, sex, disease duration and EDSS. Using the original clinical trial dataset, we extracted the following clinical variables corresponding to MRI acquisition timing: EDSS, Timed 25-Foot Walk (T25FW), 9-Hole Peg Test (9HPT), Symbol Digit Modalities Test (SDMT), and MS Walking Scale (MSWS). EDSS progression was defined as $\geq 1$ point from baseline EDSS if EDSS at entry was $\pm 5.5$ or increase of $\geq 0.5$ point if EDSS at entry was $> 5.5$. T25FW, 9HPT, SDMT and MSWS progression was defined as $\geq 20\%$ increase from baseline score.

Assessments were performed by an examining neurologist who remained unaware of the patient’s safety status and was instructed not to discuss safety issues with the treating physician, to assure an accurate and objective evaluation (Giovannoni et al., 2020).

2.6. Treatment exposure

Patients were randomised in a 1:1:1 ratio to receive oral Laquinimod in a dose of 0.6 mg or 1.5 mg or placebo (once daily). The Laquinimod 1.5 dose arm was discontinued as of January 1, 2016, due to cardiovascular side effects (patients were followed-up, but no further treatment was given). For the purpose of the current analyses, we only included patients with spinal cord MRI at baseline and week 48 visits, whilst early termination visits were excluded; for treatment effect analyses, we only included patients receiving either Laquinimod 0.6 mg or placebo.

2.7. Statistical analyses

Baseline demographics and clinical characteristics are presented as mean (and standard deviation), number (and percent), or median (and range), as appropriate.

To evaluate possible implications of different spinal cord atrophy measurements on clinical trial design (aim 1), we computed the sample size required for a hypothetical clinical trial evaluating a neuroprotective medication over a one-year period. Sample size was computed using the formula $n = \frac{\sigma^2 + \sigma^2_z}{\alpha^2} + \frac{\sigma^2_z}{\alpha^2}$, where $n$ is the required sample size per treatment arm in 1:1 randomized parallel-groups placebo-controlled trials, $Z_\alpha$ and $Z_{1-\beta}$ are kept constant (set at 5% alpha-error and 80% power, respectively), $\sigma$ is the standard deviation (from each spinal cord atrophy measurement), and $\Delta$ is the estimated treatment effect size (Cavley et al., 2018; Altmann et al., 2009). Effect size was derived from the spinal cord change for each atrophy measurement (percent change of MUCCA, CSA at C1-2 level from brain MRI, GBSI at C1-2 level from brain MRI, CSA at C1-2 level from spinal cord MRI, GBSI at C1-2 level from spinal cord MRI; CSA at C2-5 level from spinal cord MRI, and GBSI at C2-5 level from spinal cord MRI); different treatment effects were simulated (e.g., 30%, 60% and 90%). As additional estimates of measurement variability, we also computed coefficients of variation and median absolute deviations.

To evaluate the possibility of deriving longitudinal spinal cord atrophy measurements from brain MRI (aim 2), we used linear regression models including spinal cord atrophy measurements from
In smaller sample size estimates, smaller coefficients of variation and C1-2 level from spinal cord MRI; −1.5 ± 4.7% at C2-5 level from spinal cord MRI had a slightly larger rate of atrophy and a significantly pronounced longitudinal spinal cord atrophy using GBSI at C1-2 level from brain MRI (Coeff = -1.86; 95%CI = -2.44, −1.27; p < 0.01), and GBSI at C1-2 level from spinal cord MRI (Coeff = -1.47; 95%CI = -2.18, −0.75; p < 0.01) (Table 3). Patients with 9HPT progression presented with more pronounced longitudinal spinal cord atrophy using GBSI at C1-2 level from brain MRI (Coeff = -0.92; 95%CI = -1.33, −0.52; p < 0.01) (Table 3). Patients with MSWS progression presented with more pronounced longitudinal spinal cord atrophy using GBSI at C1-2 level from brain MRI (Coeff = -1.54; 95%CI = -2.83, −1.07; p < 0.01) (Table 3). No significant clinical correlates were detected for longitudinal spinal cord atrophy using MUCCA, CSA at C1-2 level from brain MRI, CSA at C1-2 level from spinal cord MRI, and CSA at C2-5 level from spinal cord MRI (Table 3).

Looking at brain cord atrophy measurements, patients with T25FWT progression presented with increased number of new T2 lesions (Coeff = 2.95; 95%CI = 0.91, 5.05; p = 0.01), T2 lesion volume change (Coeff = 266.56; 95%CI = 21.54, 511.58; p < 0.01), higher PBVC (Coeff = -0.46; 95%CI = -0.58, −0.33; p < 0.01).

3.6. Treatment effect

As previously reported (Giovannoni et al., 2020), the ARPEGGIO clinical trial failed to show any significant treatment effect on percentage brain volume changes (primary outcome measure) (Laquinimod = -0.454% and Placebo = -0.438%; p = 0.90), obtained using Structural Image Evaluation, using Normalization, of Atrophy (SIENA) (Giovannoni et al., 2020). However, Laquinimod and placebo did differ statistically in number of new T2 lesions (0.7 vs 1.6; p < 0.01), T1 lesion volume change (0.0 vs 0.0 mL; p = 0.04), and T2 lesion volume change (0 vs 3 mL; p = 0.01), whilst no differences were detected in rate of EDSS progression (17% vs 23%; p = 0.42), and change in T25FWT score (0.05 vs 0.30 s; p = 0.24). In our current post-hoc analysis, we found that no treatment effect was detected with any of spinal cord atrophy measurements (Table 4).

4. Discussion

In the present study, we explored the clinical correlates and implications for clinical trials of segmentation- versus registration-based measurements of spinal cord atrophy in progressive MS, obtained at
different cord levels from both routine brain and dedicated spinal cord MRI acquisitions. The registration-based method (GBSI) resulted in clinically meaningful measurements of spinal cord atrophy and relatively low sample sizes, and, thus, could be a candidate secondary outcome measure for clinical trials in MS. Deriving spinal cord atrophy measurements from sagittally acquired volumetric brain MRI scans using the GBSI would allow the use of spinal cord atrophy in multi-centre studies, where acquiring high quality MRI of the spinal cord may be difficult to achieve.

Overall, in line with previous studies comparing CSA and GBSI (Moccia et al., 2019; Prados et al., 2020), we confirm that GBSI measurements provide similar spinal cord atrophy rates (1.5–1.8%/year), when compared with CSA (0.9–1.5%/year) (Moccia et al., 2019; Prados et al., 2020; Casserly et al., 2018), but have smaller related standard deviations, coefficients of variation and median absolute deviations.

Table 1
Demographics and clinical features.

| Population (n = 220) |
|---------------------|
| **Age, years**      | 46.5 ± 6.8 |
| **Sex, male**       | 118 (53.6%) |
| **Baseline EDSS**   | 4.5 (3.0–6.5) |
| **Disease duration, years** | 3.4 ± 3.2 |
| **Height, cm**      | 172.0 ± 9.8 |
| **Country, n**      | 10 |

Baseline demographics and clinical features are reported for included patients.

EDSS: expanded disability status scale.

Fig. 3. Patient disposition. Patient disposition flow diagram shows number of included and excluded patients. Reasons for exclusion from the original trial population were early termination, lack of upper spinal cord segments included in brain MRI, lack of dedicated spinal cord MRI, SCT failure, GBSI failure, and implausible (> 5%) atrophy rates on both CSA and GBSI at each spinal cord level. Total number of patients with each MRI measure is presented. CSA: cross-sectional spinal cord area; GBSI: generalised boundary-shift integral; MRI: magnetic resonance imaging; SCT: spinal cord toolbox.
Fig. 4. MRI noise floor. Figure shows sagittal plane of brain and spinal cord images above (upper row) and below (lower row) the median noise floor (axial plane in the inset). Different spinal cord levels are reported (C1-2 from brain MRI, C1-2 from spinal cord MRI, and C2-5 from spinal cord MRI). Standard deviation of the MRI signal (σ) within the CSF surrounding the spinal cord is reported for each image. CSA: cross-sectional spinal cord area; GBSI: generalised boundary-shift integral; MRI: magnetic resonance imaging.

Fig. 5. Spinal cord atrophy rates by type of scan and analysis technique. Box-and-Whisker plots show annualised spinal cord percent volume change for the different measurements (percent change from MUCCA, CSA and GBSI analyses at different spinal cord levels from brain and dedicated spinal cord MRI scans). Mean, standard deviation and number of included patients are reported. CSA: cross-sectional spinal cord area; GBSI: generalised boundary-shift integral; MRI: magnetic resonance imaging; MUCCA: mean upper cervical spinal cord area.
Duetothereducedmeasurementvariability, GBSI has greater statistical power to detect (treatment) group differences. Improved performance of GBSI is likely the result of its processing pipeline, including straightening, denoising and registration of the spinal cord. In addition, GBSI boundary contours are less affected by (non-random) partial volume effects, which may bias segmentation methods (i.e., inclusion of tissue outside of the area of interest and/or exclusion of tissue within the area of interest), with improved performance (Moccia et al., 2019; Prados et al., 2020; Leung et al., 2010; Tohka, 2014).

We confirmed the strong clinical correlates of spinal cord atrophy (Brownlee et al., 2017; Ciccarelli et al., 2019). In our study, 1-year spinal cord atrophy measurements obtained with GBSI, but not with CSA, at different cord levels (C1-2 and C2-5) and from both brain and spinal cord MRI acquisitions were associated with upper and lower limb motor function, as measured by neurologists (e.g., walking test, and hand test), and by people with MS (e.g., self-reported scale on walking difficulties). Looking at previous longitudinal studies measuring spinal cord atrophy in PPMS, early changes in spinal cord area are associated with clinical changes in the long term (e.g., six to fifteen years) (Tsagkas et al., 2019; Rocca et al., 2017; Aymerich et al., 2018).

Table 2
Sample size calculations and measurement variability.

|                      | Total number of patients | Sample size effect | Coefficient of variation | Median absolute deviation |
|----------------------|--------------------------|--------------------|--------------------------|--------------------------|
|                      |                          | Treatment effect   | 30%  | 60%   | 90%   | 30%   | 60%   | 90%   | 30%   | 60%   | 90%   |
| MUCCA                | 220                      | 2073               | 518  | 230   | −3.56 | 3.06  |
| CSA at C1-2 level from brain MRI | 211  | 3454               | 864  | 384   | −4.57 | 4.13  |
| GBSI at C1-2 level from brain MRI | 181  | 832                | 208  | 92    | −2.13 | 3.26  |
| CSA at C1-2 level from spinal cord MRI | 114  | 2528               | 632  | 281   | −3.42 | 3.86  |
| GBSI at C1-2 level from spinal cord MRI | 113  | 523                | 131  | 58    | −1.83 | 2.83  |
| CSA at C2-5 level from spinal cord MRI | 114  | 1581               | 395  | 176   | −3.09 | 4.86  |
| GBSI at C2-5 level from spinal cord MRI | 113  | 1027               | 257  | 114   | −2.37 | 3.33  |

Table shows total number of patients with successfully determined measurements, the sample size estimates in each arm in a randomized clinical trial, coefficients of variation and median absolute deviations using annualised atrophy rates, and median standard deviation for each MRI measure; power was set at 80% and alpha-error at 5%. Different treatment effects were simulated (30%, 60% and 90%). No adjustment for drop-out rates due to clinical reasons or MRI quality were applied. Coefficients of variation and median absolute deviations are also reported.

CSA: cross-sectional spinal cord area; GBSI: generalised boundary-shift integral; MRI: magnetic resonance imaging; MUCCA: mean upper cervical spinal cord area.

Fig. 6. Spinal cord atrophy longitudinal changes from brain and spinal cord MRI. Scatter plots show annualised spinal cord atrophy longitudinal changes derived from brain MRI (CSA at C1-2 level from brain MRI in a and b; GBSI at C1-2 level from brain MRI in c and d) in relation to measurements obtained with the same technique but from dedicated spinal cord MRI (CSA at C1-2 level and CSA at C2-5 level from spinal cord MRI in a and b; GBSI at C1-2 level and GBSI at C2-5 level from spinal cord MRI in c and d). β-Coefficients (Coeff), p-values and 95% confidence intervals (95%CI) are reported from linear regression models. CSA: cross-sectional spinal cord area; GBSI: generalised boundary-shift integral; MRI: magnetic resonance imaging.
Table 3: Clinical correlates of longitudinal spinal cord atrophy.

| Variable                        | Yes (23.9%) | No (76.1%) |
|---------------------------------|-------------|------------|
| MUCCA −1.03 ± 3.59              | −0.82 ± 3.94| −1.33 ± 3.02|
| CSA at C1-2 level from brain MRI| −0.93 ± 4.22| −1.35 ± 4.16|
| GBSI at C1-2 level from brain MRI| −1.77 ± 3.48| −1.25 ± 3.18|
| GBSI at C2-5 level from spinal cord MRI| −1.84 ± 2.86| −0.96 ± 2.72|
| Table shows longitudinal spinal cord atrophy in relation to the progression of different clinical outcomes. Percent of patients progressing on each clinical outcome is also shown. * indicates p < 0.05 on different stepwise logistic regression models and different MRI variables in the model. MUCCA: mean upper cervical spinal cord area; CSA: cross-sectional spinal cord area; GBGI: generalised boundary-shift integral; MRI: magnetic resonance imaging. |
increased from 43 on C1-2 level from brain MRI, to 57 and 76 on C1-2 and C2-5 levels on spinal cord MRI, respectively). As such, obtaining spinal cord atrophy measurements from brain scans could represent a viable, efficient and clinically meaningful alternative to the more technically challenging spinal cord image acquisition, in particular in multi-centre settings where homogenous quality of spinal cord acquisitions is difficult to achieve. So far, the possibility to derive spinal cord measurements from brain scans has been successfully explored only in cross-sectional studies (Prados and Barkhof, 2018; Liu et al., 2016; Tsagkas et al., 2018). In our study, notwithstanding the large variability of centres acquiring images with different expertise, protocols and field strength, we found statistically significant, but small, correlation coefficients between measurements derived from brain and cord scans, being higher for GBSI than CSA (0.3 vs 0.2), suggesting that longitudinal spinal cord atrophy from brain scans could better be derived using GBSI than using segmentation-based approaches (e.g., CSA). Differences in noise floor between brain and spinal cord MRI could explain the partial concordance of measurements and could be due to the use of different coils, that can affect image quality at C1-2 level. Indeed, more advanced coils, optimised for both brain and spinal cord, can improve image quality, especially at upper cervical cord level, when compared with conventional coils (Cohen-Adad et al., 2011). Unfortunately, the ARPEGGIO database did not include sufficiently detailed information on coil design at different sites to be accounted for in the statistical models.

Sample size estimates with spinal cord GBSI (e.g., 130–250 per treatment arm, for a 60% effect) are two-to-four fold smaller than CSA (e.g., 400–800 per treatment arm, for a 60% effect), and are on the same order of magnitude as for brain atrophy (the pooled rate of 1-year brain atrophy in placebo- and Laquinimod-treated patients from the ARPEGGIO trial (-0.45 ± 1.00%) corresponds to 215 patients per treatment arm for a 60% treatment effect) (Giovannoni et al., 2020; Moccia et al., 2019). In our previous study on spinal cord GBSI (Moccia et al., 2019), we obtained even smaller estimates for both GBSI (50–100), and CSA (200–800), considering a similar treatment effect and also accounting for physiological spinal cord loss in healthy controls. However, that previous study was conducted in much more selected centres, using MRIs acquired within the Magnetic Resonance Imaging in MS (MAGNIMS) network (Moccia et al., 2019). In the present study, we tried to simulate similar conditions by classifying scans based on the median noise floor; however, when including top quality scans, atrophy variability only partly improved, with increased effect size and reduced standard deviation for some measurements. Thus, future clinical trials aiming to use spinal cord atrophy as a primary outcome measure, rather than simply increase the sample size to account for drop-out rates during analyses, should focus on sites that are able to acquire high quality images. A consensus spinal cord acquisition protocol has been recently developed and tested in about 30 different sites across the world, including different vendors (GE, Siemens and Philips) and could serve as a standard for future multicentre spinal cord MRI studies (http://www.spinalcordmri.org – Protocols). Alternatively, longer follow-up could also be considered (Altmann et al., 2009); unfortunately, in the ARPEGGIO trial, no MRI scans were available beyond week 48.

Finally, we confirmed the lack of treatment effect for Laquinimod on spinal cord atrophy, as in the original ARPEGGIO analysis (Giovannoni et al., 2020). This finding, also in light of the lack of treatment effect on brain atrophy and on disability outcomes (Giovannoni et al., 2020), points towards poor (neuroprotective) efficacy of Laquinimod, rather than measurement issues.

Limitations of the present study include the relatively short follow-up duration (48 weeks). On the one hand, this is in line with phase 2 clinical trial requirements in MS where brain atrophy is the primary outcome measure. However, one-year follow-up is relatively short to acquire sufficiently reliable clinical outcomes (e.g., lack of association with EDSS progression), that could have been better studied in the long term. Also, we used DeepSeg for spinal cord segmentation, which is an automated method with high reproducibility, but providing smaller volumes than other methods (e.g., NeuroQLab and Jim) (McCoy et al., 2019; Weeda et al., 2019); we previously ruled out the impact of different segmentation methods on longitudinal spinal cord atrophy measurements on GBSI, but cannot exclude results using the segmentation-based method (CSA) were affected (Prados et al., 2020). Also, we used the standard deviation of the MRI signal within the ring of CSF surrounding the spinal cord to classify scans based on their noise floor (Jones and Basser, 2004; Tabelow et al., 2015), which is a common approach for low signal situations, such as for CSF on T1-weighted images (Prados et al., 2020; Jones and Basser, 2004). This was automatically computed by our pipeline, and represents the variance of the background/CSF signal, independent from its actual scale; other measures (e.g., contrast-to-noise ratio) could be considered in the future, but would require additional manual intervention, which is undesirable for an automated pipeline aiming to be applied in large datasets. Finally, spinal cord acquisitions in ARPEGGIO only included T1 sequences, where outlining lesions is difficult; in the future, the possibility of using PSIR and T2 lesions for GBSI calculation will allow spinal cord lesion segmentation and filling, which can make cord atrophy estimates less variable, as has been shown for brain atrophy (Amiri et al., 2018; Battaglini et al., 2012; Popescu et al., 2014).

### 5. Conclusions

Imaging the spinal cord is challenging, but clinically relevant. Spinal cord atrophy reflects early subtle changes in motor function of the upper and lower limbs, with possibly more obvious clinical correlates in the long-term. Improvements in spinal cord acquisition, processing and analysis (e.g., SCT and GBSI), along with the possibility of deriving spinal cord atrophy measurements from brain MRI scans, can enhance the application of this clinically meaningful imaging outcome measure in multi-centre longitudinal observational studies and clinical
trials. Rigorous quality control will be required though, as dedicated spinal cord scans (and the resulting measurements) remain more variable than brain scans. Despite sample size not being far off from those for brain atrophy in the ARPEGGIO study, spinal cord atrophy is destined to remain a secondary outcome for the moment, given the issues around controlling image quality in the spinal cord.

CRediT authorship contribution statement

**Marcello Moccia:** Conceptualization, Data curation, Formal analysis, Software, Validation, Writing - original draft.

**Nicola Valsecchi:** Data curation, Formal analysis, Visualization, Writing - original draft.

**Olga Ciccarelli:** Funding acquisition, Investigation, Project administration, Resources, Writing - review & editing.

**Ronald Van Schijndel:** Data curation, Investigation, Methodology, Software, Writing - review & editing.

**Frederik Barkhof:** Conceptualization, Funding acquisition, Resources, Supervision, Writing - review & editing.

**Ferran Prados:** Conceptualization, Formal analysis, Methodology, Software, Validation, Writing - original draft.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

- MM has received research funding from ECTRIMS-MAGNIMS, the UK MS Society, and honoraria from Biogen, Merck, Novartis, Roche and Teva; and consulting fees from Veterans Evaluation Services. NV, and RVS have nothing to disclose. OC has received research funding from the Netherlands Organisation for Health Research and Development (ZonMw) and honoraria from Bayer-Schering Pharma, Biogen-Idec, Teva, Merck-Serono, Novartis, Roche, Synthon BV, Jansen Research, Genzyme, and IXICO. FP has received research grants from Guantivors of Brain, and honoraria from Bioclinica.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2020.102418.

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