Evaluation of Intra- and Interscanner Reliability of MRI Protocols for Spinal Cord Gray Matter and Total Cross-Sectional Area Measurements

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**Background:** In vivo quantification of spinal cord atrophy in neurological diseases using MRI has attracted increasing attention.

**Purpose:** To compare across different platforms the most promising imaging techniques to assess human spinal cord atrophy.

**Study Type:** Test/retest multisscanner study.

**Subjects:** Twelve healthy volunteers.

**Field Strength/Sequence:** Three different 3T scanner platforms (Siemens, Philips, and GE) / optimized phase sensitive inversion recovery (PSIR), T1-weighted (T1-w), and T2*-weighted (T2*-w) protocols.

**Assessment:** On all images acquired, two operators assessed contrast-to-noise ratio (CNR) between gray matter (GM) and white matter (WM), and between WM and cerebrospinal fluid (CSF); one experienced operator measured total cross-sectional area (TCA) and GM area using JIM and the Spinal Cord Toolbox (SCT).

**Statistical Tests:** Coefficient of variation (COV); intraclass correlation coefficient (ICC); mixed effect models; analysis of variance (t-tests).

**Results:** For all the scanners, GM/WM CNR was higher for PSIR than T2*-w ($P < 0.0001$) and WM/CSF CNR for T1-w was the highest ($P < 0.0001$). For TCA, using JIM, median COVs were smaller than 1.5% and ICC >0.95, while using SCT, median COVs were in the range 2.2–2.75% and ICC 0.79–0.95. For GM, despite some failures of the automatic segmentation, median COVs using SCT on T2*-w were smaller than using JIM manual PSIR segmentations. In the mixed effect models, the subject was always the main contributor to the variance of area measurements and scanner often contributed to TCA variance ($P < 0.05$). Using JIM, TCA measurements on T2*-w were different than on PSIR ($P = 0.0021$) and T1-w ($P = 0.0018$), while using SCT, no notable differences were found between T1-w and T2*-w ($P = 0.18$). JIM and SCT-derived TCA were not different on T1-w ($P = 0.66$), while they were different for T2*-w ($P < 0.0001$). GM area derived using SCT/T2*-w versus JIM/PSIR were different ($P < 0.0001$).

**Data Conclusion:** The present work sets reference values for the magnitude of the contribution of different effects to cord area measurement intra- and interscanner variability.

**Level of Evidence:** 1

**Technical Efficacy:** Stage 4

Quantifying spinal cord atrophy and the more recently described spinal cord gray matter atrophy in various neurologic conditions including trauma, inflammation, or neurodegeneration has gained increasing attention, particularly with the development of dedicated spinal cord imaging techniques.1–6 Spinal cord dedicated volumetric 3D T1-weighted (T1-w) protocols, similar to the ones widely used for brain volume estimation, are becoming a standard for total cross-sectional area (TCA) measurements.7 However, on images acquired with this and other conventional T1-w and T2-weighted (T2-w) protocols, the gray matter (GM) / white matter (WM) contrast is suboptimal to allow separate assessment of these two tissues.

The most promising imaging techniques used so far to measure GM area/volume in the spinal cord are based on...
The goal of this study was to perform direct comparisons of three selected optimized protocols for spinal cord GM and TCA segmentation on the same group of 12 healthy controls, on three 3T scanners produced by the three main vendors of human magnetic resonance imaging (MRI) scanners: Siemens, Philips, and General Electric (GE). The chosen protocols used product sequences available on all systems.

The two operators manually drew a GM ROI on the anterior part of the GM (an area spanning the anterior horns). Three WM ROIs were symmetrically drawn in the region of the lateral and posterior columns. Two CSF ROIs were symmetrically placed in the right and left spinal canal. The same group of ROIs was used for all the acquisitions of a subject. Examples of ROIs are reported in Figs. 1, 2 and 3.

CNR between tissues 1 and 2 was computed for each subject, scanner, and protocol as previously defined:

\[ \text{CNR}_{12} = \frac{\text{SI}_1 - \text{SI}_2}{\sqrt{(\text{SD}_1^2 + \text{SD}_2^2)}} \]  

where \(\text{SI}_1\), \(\text{SI}_2\), \(\text{SD}_1\), and \(\text{SD}_2\) respectively indicate the mean intensity value within the tissues 1 and 2 ROIs, and the corresponding standard deviations.

Average values and SDs between operators were computed and differences between scanners and protocols tested using two-tailed t-tests (\(P < 0.05\)).
To estimate intra- and interscanner reliability of area measurements, TCA and GM were computed for all the available images acquired with the different protocols.

In order to assess the potential impact of the segmentation method on the spinal cord metrics, we selected the two most widely used approaches based on recent literature. The first approach is JIM v6 (Xinapse Systems, http://www.xinapse.com) that was previously used in different studies.1,3,11,12,19–25 The second approach is the open source Spinal Cord Toolbox (SCT) (https://sourceforge.net/p/spinalcordtoolbox/wiki/Home/) that has seen recent utilization.14,23,26–33

While the methods based on JIM have been previously optimized and tested for GM/TCA segmentation of single-slice 2D PSIR images and for TCA extracted by 3D T1-w and T2/T2* − w images, the SCT has been optimized and tested for TCA extraction from T1-w/T2-w acquisitions and for GM/TCA segmentation of T2* − w images.

| TABLE 1. 2D Phase Sensitive Inversion Recovery (PSIR), 3D T1-w, and 2D T2*−w Protocol Parameters |

|               | Siemens | Philips | GE       |
|---------------|---------|---------|----------|
| Sequence name |         | T1-TFE  | PSMDE    |
| Dimension     | 2D      | 2D      | 2D       |
| TR (msec)     | 4000    | 9.5     | 8.00     |
| TE (msec)     | 3.22    | 4.7     | 3.76     |
| TI (msec)     | 400     | 300     | 400      |
| # averages    | 3       | 5       | 20       |
| Shots         | 9       | 10      | 52       |
| Segments      | 26      | 24      | 4/5      |
| Flip angle (deg) | 10    | 15      | 25       |
| Voxel size (mm) | 0.78 × 0.78 × 5 | 0.78 × 0.78 × 5 | 0.78 × 0.78 × 5 |
| Field of view (mm) | 200 × 200 × 5 | 200 × 200 × 5 | 200 × 200 × 5 |
| BW(Hz/Px)     | 250     | 151.7   | 113.6 (22.73 kHz tot) |
| Phase encoding dir. | R > > L | R > > L | A > > P |
| Parallel acc. factor | no | no | no |
| Acq. time (min:sec) | 1:52  | 2:30   | ~3 (dep. on heart rate) |
| Cardiac gating | simulated | not needed | finger pulse |
| Orientation   | axial   | axial   | axial    |

| 3D T1-w       | Siemens | Philips | GE       |
|---------------|---------|---------|----------|
| Sequence name | MPRAGE  | T1-TFE  | BRAVO    |
| Dimension     | 3D      | 3D      | 3D       |
| TR (msec)     | 2000    | 7.77    | 8.71     |
| TE (msec)     | 3.72    | 3.56    | 3.66     |
| TI (msec)     | 1000    | 1000    | 450      |
| # averages    | 1       | 1       | 2        |
| Flip angle (deg) | 9      | 8       | 12       |
| Voxel size (mm) | 1 × 1 × 1 | 1 × 1 × 1 | 1 × 1 × 1 |
| Field of view (mm) | 320 × 260 × 192 | 256 × 256 × 192 | 256 × 256 × 96 |
acquisitions with multiple slices/volumetric coverage. Therefore, JIM was used to segment TCA on the T1-w, PSIR, and T2* -w images, and to segment GM on PSIR images. The automatic SCT was used to segment TCA on T1-w and T2* -w images, and to segment GM on T2* -w images. We did not use the SCT on the single-slice 2D PSIR images, because the SCT is optimized for multiple slices/volumetric coverage, and JIM for the GM segmentation of T2* -w images, because we believe there are no exhaustive published data regarding the reliability of manual segmentations with this combination of software/contrast.

2D PSIR IMAGES. TCA and GM areas for each participant and scanner were measured on the phase-sensitive reconstructed images. TCA estimates were obtained in a semiautomated way using an active surface model34 available in JIM, with a method previously shown to have high intra- and inter-rater reliability.1,11 Briefly, this was done using the cord finder toolkit with fixed settings (nominal cord diameter 8 mm, number of shape coefficients 24, order of longitudinal variation 12). The marker requested by the toolkit was positioned by a single experienced operator (NP) on the mid-sagittal WM, directly posterior to the gray commissure.

GM areas were manually measured using JIM with a segmentation technique that has been shown to be highly reliable. GM area was segmented three times using JIM by NP for each participant and scanner. The average GM area obtained from the three segmentations was finally calculated.1,11 From previous experience1,3,11,35,36 the interoperator variability of the segmentations performed with the JIM methods are expected to have coefficient of variation (COV) <0.5% and intraclass correlation coefficient (ICC) >0.99 for the TCA semiautomated measurements, and COV in the range 3–5% and ICC ~0.90 for the GM area manual measurements.

T1-W AND T2*-W IMAGES. Two methods were used to calculate TCA on T1-w and T2*-w acquisitions. The first

| TABLE 1. Continued |
|---------------------|
| **3D T1-w**        |
| Siemens      | Philips   | GE          |
| BW (Hz/Px)  | 150       | 191.5       | 97.65 (25.00 kHz tot) |
| Phase encoding dir. | A >> P   | A >> P      | R >> L        |
| Parallel acc. factor | 2        | 2           | no            |
| Acq. time (min:sec) | 4:44     | 4:56        | 7:28          |
| Orientation   | sagittal  | sagittal    | sagittal      |

| **2D T2*-w**       |
|---------------------|
| Siemens      | Philips   | GE          |
| Sequence name | MEDIC     | M-FFE       | MERGE        |
| Dimension    | 2D        | 2D          | 2D           |
| TR (msec)    | 627       | 625         | 777          |
| TE (msec)    | 15        | 2.52        | 13.77        |
| # echoes     | 3         | 3           | 3            |
| # averages  | 2         | 1           | 2            |
| Flip angle (deg) | 30       | 30          | 30           |
| Voxel size (mm) | 0.5 × .0.5 × 3 | 0.5 × .0.5 × 3 | 0.5 × .0.5 × 3 |
| Field of view (mm) | 160 × 160 × 45 | 160 × 160 × 45 | 128 × 128 × 45 |
| BW (Hz/Px)  | 240       | 241.1       | 195.31 (25.00 kHz tot) |
| Phase encoding dir. | R >> L  | R >> L      | A >> P       |
| Parallel acc. factor | 2        | no          | no           |
| Acq. time (min:sec) | 3:52     | 4:02        | 6:44         |
| Orientation   | axial     | axial       | axial        |
method, semiautomated, was used in previous publications.\textsuperscript{3,19,20} TCA on T1-w images was measured by reslicing the sagittal acquisitions and extracting five consecutive 1-mm-thick axial slices perpendicular to the long axis of the cord at the C2-C3 disc level, and measuring the average area of the cord using the semiautomated cord finder toolkit of JIM with the same fixed settings used for PSIRs. The markers requested by the toolkit were placed at the center of the spinal cord in each of the five slices. For T2*-w images a similar process was applied to a single axial slice at the C2-C3 disc level without any reslicing.

The second method used the fully automatized SCT. Original images were preprocessed in the native space and then registered to the PAM50 spinal cord template.\textsuperscript{31} The TCA was extracted from T1-w and T2*-w scans following automatic cord segmentation (using in order the commands “sct_propseg,” “sct_label_vertebrae,” “sct_label_utils,” “sct_register_to_template,” and “sct_warp_template”\textsuperscript{33} with default parameters, following the documentation available at https://sourceforge.net/p/spinalcordtoolbox/wiki/tools/) and then averaged within the C3 vertebra automatically labeled by the software (command “sct_process_segmentation”). The GM area on T2*-w images was extracted with the SCT following automatic gray matter segmentation (command “sct_segment_graymatter”)\textsuperscript{32} and extracting the averaged value within the C3 vertebra (command “sct_process_segmentation”).

Statistical Analysis

All statistical analyses were performed using JMP Pro 13 (SAS Institute, Cary, NC).

- The coefficient of variation (COV = 100 × (absolute difference / mean of measurements)) for all the test/retest couples of TCA and GM area measured with the different segmentation methods/protocols was calculated and its median/mean (SD) on the group of subjects computed for each scanner.
- The ICC was calculated between all the test/retest couples of TCA and GM area measured, for each different segmentation method/protocol, and for each scanner.
- Bland–Altman plots were produced for each of the combinations segmentation method/protocol, representing each scanner with a different symbol. On the Bland–Altman plots, the difference of the retest and test measurements was reported on the y-axis and their mean value on the x-axis.
- Mixed models with scanner as fixed effect, and test–retest and subject as nested random effects were used to estimate the contribution of subject, test–retest acquisition, and scanner to the variance of obtained measures.
- To visualize interscanner differences in the calculated areas, the average values between test and retest acquisitions were computed and graphed for each of the combinations method/protocol.
- To evaluate the effect of acquisition protocol when using the same segmentation method, analysis of variance (ANOVA) (t-tests) were used between couple of measurements for TCA (PSIR, T1-w, and T2*-w images segmented with JIM and T1-w and T2*-w images segmented with SCT). ANOVA was used also to evaluate the effect of the segmentation method on the same protocol (T1-w images and T2*-w images segmented with JIM and SCT). Finally, ANOVA was used to see if there were statistically significant differences (P < 0.05) in the GM area values obtained with the two couples segmentation method/protocol (SCT/T2*-w vs. JIM/PSIR).

Results

Qualitative Quality Assessment

All images acquired with PSIR and T2*-w protocols at the C2-C3 disc level for the 12 healthy controls on the three different scanners are reported in Figs. 1 and 2, demonstrating overall good quality of both the PSIR and T2*-w images. Overall quality consensus scores for PSIR were 24 for all the scanners, while for T2*-w images they were 23, 18, and 22, respectively for Siemens, Philips, and GE.

Consensus visual qualitative assessment scores suggest that the PSIR images on the GE scanner appeared slightly more noisier/affected by motion (22) compared with the other two scanners (24), while according to this score, the T2*-w images appeared to be of a slightly better quality on Siemens (23) and GE (24) scanners compared with Philips (22). With regard to the GM delineation/contrast, visual qualitative assessment indicated consistent good quality for the PSIR images (23, 24, 23 for Siemens, Philips, and GE), while the quality of some of the T2*-w images (12 over 72, which means about 17%) was suboptimal (scores 24, 19, 23 for Siemens, Philips, and GE).

Illustrative images acquired on a single subject with the T1-w protocol are shown in Fig. 3. The quality of T1-w images was in general consistent with the reported example and consistent across the different scanners (overall quality scores and noise/motion were 24, 24, and 23, respectively, for Siemens, Philips, and GE). We excluded from the following analyses two T1-w acquisitions because the subject clearly moved during the acquisition (both were test scans on the GE scanner).

CNR Evaluation

In Table 2 the GM/WM and WM/CSF CNR measured at the C2-C3 disc level for PSIR, T2*-w, and T1-w protocols is reported.

The GM/WM CNR for the PSIR protocol was higher compared with the T2*-w protocol for all the scanners (P < 0.0001). GM/WM CNR for the T2*-w protocol on the
Philips scanner was lower if compared with Siemens \((P < 0.0001)\) that was lower if compared with GE \((P < 0.0001)\), confirming the visual qualitative impression of slightly worse quality on Philips. The visual impression that the PSIR images were noisier on GE, however, is not supported by the CNR evaluation.

The WM/CSF CNR for the T1-w protocol was consistently higher for all the scanners compared with the PSIR and T2*−w protocol images \((P < 0.0001)\). The WM/CSF CNR for PSIR and T2*−w protocols was comparable for GE \((P = 0.06)\) and Philips \((P = 0.042)\), while for Siemens for PSIR it was much higher \((P < 0.0001)\).

**TCA and GM Area Measurements**

Test–retest COVs and ICC for measured TCA and GM area for all the combinations of segmentation methods and protocols are reported in Table 3.

For the TCA, with the JIM semiautomatic method, median COVs are very similar across the three protocols and smaller than 1.5% (with the only exception of PSIR on the GE scanner). ICC were always >0.95. The SCT performed very similarly when measuring TCA on T1-w and T2*−w protocols for all the scanners, with median COV in the range 2.2–2.75% and ICC in the range 0.79–0.95.
With regard to GM, median COV of measurements obtained with the SCT on $T_2^*$-w images were consistently smaller compared to manual segmentation on PSIR images. Nevertheless, mean and SD of COV for the SCT/$T_2^*$-w combination are in general larger than on JIM/PSIR, because of outlier values due to failures of the automatic segmentation algorithm (Bland–Altman plots reported in Fig. 4). The lowest ICC were 0.3423 for SCT/$T_2^*$-w on GE and 0.6915 for JIM/PSIR on Philips.

Furthermore, despite similar COV median values, mean/SD are bigger (and ICC smaller) for SCT on $T_2^*$-w images than for $T_1$-w images, due to fewer automatic segmentation errors of the SCT on $T_1$-w images.

**Statistical Analysis**

In the mixed effect models, subject was always the main contributor to the variance of the area measurements (always significant, Wald $P < 0.05$). Among all the combinations of segmentation methods/protocols/tissues, session (test–retest) was statistically significant in explaining the variance of the area measurements only for TCA measured on $T_1$-w images with the SCT ($P < 0.0001$). Scanner (fixed effect) was instead
often statistically significant in the mixed effect models for TCA measurements. The value of the estimated intercept (that can be read as values for Siemens for the way the variables were ordered in the model), the biases of the measurements that the model attributes to the other scanners and the related \( P \)-values are reported in Table 4.

The mean value of the test and retest acquisitions for the 12 healthy subjects are reported in Fig. 5 for each segmentation method/protocol/area.

According to the ANOVA, there was a statistically significant difference for TCA measurements on the \( T_2^*\)-w protocol segmented with JIM, compared to both PSIR (\( P = 0.0021 \)) and \( T_1\)-w (\( P = 0.0018 \)). No difference was found between the \( T_1\)-w and \( T_2^*\)-w protocols when the segmentation was performed using the SCT (\( P = 0.18 \)).

Regarding the difference attributable to the segmentation method on the same protocol for TCA measurement, on \( T_1\)-w images JIM and SCT were not statistically different (\( P = 0.66 \)), while there was difference for the \( T_2^*\)-w contrast (\( P < 0.0001 \)).

Finally, there were very significant differences in the GM area obtained using the SCT/\( T_2^*\)-w vs. JIM/PSIR segmentation method/protocol combinations (\( P < 0.0001 \)). However, no scanner-related bias was detected for GM areas.

All results are graphed in Fig. 6, where \( P \)-values for the comparisons between different combinations of segmentation method/protocol are also reported.

**Discussion**

In this work we present for the first time analysis of a rich MRI dataset, acquired on the same 12 healthy subjects on three 3T scanners produced by the main commercial brands with the most promising protocols for TCA and GM area assessments. A qualitative assessment and quantitative evaluation of CNR and intra- and interscanner reliability of area measurements at the C2-C3 spinal cord level is presented.

For GM delineation, the quality of PSIR images was more consistent than \( T_2^*\)-w images. These qualitative visual impressions were confirmed by CNR evaluations that showed that GM/WM CNR for PSIR images was higher than for \( T_2^*\)-w images for all the scanners. The observed tendency for fuzzy appearance of GM on \( T_2^*\)-w compared to PSIR images may be the result from higher sensitivity of the \( T_2^*\)-w protocol to susceptibility artifacts and motion.

**TABLE 2.** Between Operators Mean (Standard Deviation) Contrast-to-Noise Ratio (CNR) Between Gray Matter (GM) and White Matter (WM) Tissues, and Between WM and Cerebrospinal Fluid (CSF), for the Three Different Acquisition Protocols (PSIR, \( T_2^*\)-w and \( T_1\)-w) on the Three Different Scanners

| CNRGM/WM | PSIR | \( T_2^*\)-w | \( T_1\)-w |
|----------|------|-------------|-----------|
| Siemens  | 2.11 (0.15) | 1.56 (0.06) | —         |
| Philips  | 3.14 (0.16) | 1.09 (0.05) | —         |
| GE       | 2.39 (0.04) | 1.67 (0.02) | —         |

| CNRWM/CSF | PSIR | \( T_2^*\)-w | \( T_1\)-w |
|-----------|------|-------------|-----------|
| Siemens   | 8.45 (0.22) | 3.54 (1.38) | 9.91 (0.14) |
| Philips   | 3.06 (0.11) | 3.71 (1.05) | 9.08 (0.04) |
| GE        | 3.40 (0.07) | 4.64 (2.16) | 7.26 (0.05) |
3D T1-w images were consistently of good quality for all the scanners in terms of spinal cord/CSF delineation. This is not surprising, considering that the used protocols are optimizations of 3D inversion recovery spoiled gradient echo protocols that have become a standard for atrophy assessment on brain images over more than a decade.

The goal of having comparable CNR across different vendors was overall achieved with the chosen sequences/parameters/hardware, with in general 3D T1-w protocols giving higher WM/CSF CNR than the other two protocols and PSIR higher GM/WM CNR than T2*-w protocols. It has to be mentioned that, since there were different hardware configuration/protocol choices, we preferred not to correct CNR for acquisition times/coverage when evaluating CNR at the C2-C3 level. It has also to be mentioned that different resolutions can affect the CNR.

For TCA estimates, all protocols performed very similarly in terms of intra- and interscanner reliability, for a given segmentation method. The semiautomatic method based on JIM showed better test–retest COV and ICC on both 3D T1-w and T2*-w protocols compared with the automatic SCT method.

We therefore think there is not an obvious choice of best protocol if the goal of a study is TCA evaluation. The choice has to be driven by a series of factors and considerations such as the acquisition time that can be spent on a protocol, the spinal cord levels to be covered, the specific hardware available, and the need of assessing TCA alone or in combination with GM area measurements.

### TABLE 3. Test-Retest COV (Median, Top Row, and Mean (SD), Middle Row) and ICC (Bottom Row) for TCA and GM Area Measurements on Images Acquired With the Three Protocols (PSIR, T1-w, T2*-w) and Using the Different Segmentation Methods (JIM, SCT) on the Group of 12 Controls

| Method | Siemens | Philips | GE |
|--------|---------|--------|----|
| TCA    |         |        |    |
| JIM TCA T1-w | 0.84 (1.25) | 1.16 (1.32) | 1.02 (0.96) |
| JIM TCA PSIR | 1.09 (1.40) | 1.14 (0.83) | 2.38 (1.53) |
| JIM TCA T2*-w | 0.9892 | 0.9828 | 0.9515 |
| SCT TCA T1-w | 2.27 (2.35) | 1.36 (1.90) | 0.82 (0.96) |
| SCT TCA PSIR | 2.53 (2.16) | 3.55 (2.74) | 1.15 (0.96) |
| SCT TCA T2*-w | 0.9671 | 0.9720 | 0.9903 |
| SCT GM T2*-w | 5.87 (12.96) | 3.91 (4.04) | 5.60 (7.14) |
| SCT GM PSIR | 0.8121 | 0.8652 | 0.8770 |

Data for each scanner are reported.

### FIGURE 4: Bland–Altman plots reporting the difference between the TCA and GM area retest and test measurements (y-axis) and their mean value (x-axis), for all the combinations of software and protocol.
GM as well. It is worth noting that $T_2^*\text{-}w$ protocols gave significant biases in comparison to 3D $T_1\text{-}w$ and PSIR protocols for TCA estimates when JIM segmentation was used.

When using JIM, biases across scanners tended to be statistically more significant than when using the SCT; the test–retest COV and biases across scanners with JIM measurements were all bigger than the interoperator variability reported in the previous literature. The statistical sensitivity to scanner model for JIM could be explained considering the lower interoperator and intrascanner variability of JIM-based segmentations, thereby providing statistical power to detect small biases across scanners.

GM segmentations were performed with the method/protocol couples JIM/PSIR and SCT/$T_2^*\text{-}w$. The latter combination gave lower median COVs. There was a statistically significant large bias between values obtained with the two segmentation method/protocol combinations. $T_2^*\text{-}w$ derived GM areas were much smaller than PSIR derived ones. This could be due to the contrast difference or the higher resolution for the $T_2^*\text{-}w$ protocols. It has been shown previously that higher resolution PSIR gives smaller partial volume effects on the WM/GM edge and therefore smaller GM area estimates.$^{37}$

Automatic methods have obvious advantages if compared to manual or semiautomatic methods, but if they often need corrections they are essentially semiautomatic methods. The SCT was not robust as an automatic method on these spinal cord data and had higher failure rates on $T_2^*\text{-}w$ images than $T_1\text{-}w$ images. This explains the low median values, but bigger mean and standard deviations in some cases. In a few cases, the SCT repeated the same error for both test and retest acquisitions of a particular subject, in particular for $T_2^*\text{-}w$ images (for example, wrong vertebra assignment, or wrong total cord delineation and subsequently GM segmentation). These systematic errors gave a good intrascanner reproducibility of GM area, but it was evident with a visual check that the segmentations were not accurate in both the test and retest acquisitions. We also noticed that the SCT provided a GM segmentation result even when there was no WM/GM delineation (a suboptimal $T_2^*\text{-}w$ image, but even on a 3D $T_1\text{-}w$ images with no GM visible). The SCT can be very useful also in these situations to create a probabilistic GM mask to be used to calculate metrics on other acquisitions/contrasts, but it could give misleading information if used to quantitatively assess the GM area.

These observations could explain why in a published work that tested the SCT GM segmentation method and other methods, the Dice Similarity Coefficients and Jaccard Index indicated moderate overlap of segmentations obtained with SCT with gold-standard manual segmentations.$^{15}$

Manually correcting SCT errors was beyond the scope of the present work, but there is clearly room for improving
this very useful tool, maybe tuning it to the different specific acquisitions. Manual and semiautomatic methods have the disadvantage of being time-consuming and can have high interrater variability. Nevertheless, the semiautomatic method based on JIM consistently gave better reliability for TCA estimates on all the tested protocols. For the GM manual segmentations performed with JIM, the observed test–retest COVs were of the same order of magnitude of the interoperator variability previously reported. The statistical power provided by the GM segmentation technique was therefore not sufficient to disentangle all the possible sources of variability in the measurement (scanner, positioning at acquisition, test–retest, interoperator variability of segmentations).

A limitation of this study is that software/hardware varied across scanners. For example, the available GE scanner did not have anterior neck coils, which prohibited the use of parallel imaging. For this reason, the implementation of some protocols on GE was a little different than on Siemens and Philips, where we managed to set more similar protocols. The T1-w sequence on GE had a smaller field of view (FOV) compared to the other scanners, different bandwidth, but two averages were made to compensate for the CNR lost. Different FOVs can affect the quality of shimming and the CNR, and therefore affect the quality of segmentations. A different choice could have been made; for example, an IR-SPGR sequence could have been used instead of BRAVO, or increasing the FOV instead of making two averages. Analogous differences due to the lack of parallel acceleration capability were present in the T2*–w protocol. Also for PSIR, the way the different vendors implement the cardiac gating in the protocol forced differences in the settings on the different scanners.

Another limitation of this study is that we performed analyses with only two segmentation methods. We also decided not to perform segmentation for every protocol/segmentation method combination but constrained our analyses to only those applications already shown to be appropriate for the given segmentation method. Further developments in segmentation methods may help to reduce the variability in the area estimates. These data could be used for testing (and possibly improving) other algorithms.

Other limitations are the absence of a T2-w protocol, the limited number of subjects, and the fact that only data on healthy subjects were acquired (all choices forced by the very demanding protocol that required an hour of scan on three different scanners in a very short time frame).

![FIGURE 6: ANOVA for TCA and GM area measured with the different combinations segmentation method/protocol. P-values for the different couples of comparisons are reported and highlighted in bold when differences were statistically significant (P < 0.05).](image)

### TABLE 4. Mixed Effect Model Results

|         | JIM TCA T1-w | JIM TCA PSIR | JIM TCA T2*-w | SCT TCA T1-w | SCT TCA T2*-w | SCT GM T2*-w | JIM GM PSIR |
|---------|--------------|--------------|---------------|--------------|--------------|--------------|-------------|
| Siemens (intercept) | 79.94 | 80.63 | 84.62 | 78.81 | 77.49 | 13.90 | 20.28 |
| Philips  | –0.62 | 0.37 | –1.54 | 0.82 | 0.26 | 0.04 | 0.09 |
|          | P = 0.0012 | P = 0.25 | P < 0.0001 | P = 0.13 | P = 0.74 | P = 0.81 | P = 0.48 |
| GE      | –0.56 | –1.42 | 0.64 | –1.59 | 0.84 | 0.24 | 0.08 |
|          | P = 0.0039 | P < 0.0001 | P = 0.016 | P = 0.0047 | P = 0.28 | P = 0.15 | P = 0.53 |

Estimated scanner contribution to biases: Estimated intercept (corresponding to Siemens) and the bias and related P value for the other scanners are reported (in bold when < 0.05). Values of areas are in square mm.
While there may be further room for optimization, we believe that our efforts reflect the expected biases and variability due to the choice of scanners, protocols, and segmentation methods. The present work suggests that multiscanner/multicenter studies for TCA/GM segmentation are feasible with all the techniques explored in the study.

This study may set reference values for the magnitude of the contribution of different effects (scanner, protocol, segmentation method) to TCA/GM area measurement intra- and interscanner variability. The data and results reported in the present study may help in making informed decisions when planning a specific study, depending on the different acquisition settings and study goals.

Further optimization of protocols and segmentation algorithms is warranted and this study can help in determining what are the directions in which the spinal cord MRI community should move along.

Acknowledgments

First and foremost, the authors would like to thank the twelve subjects who participated in this study. Repeating the MRI acquisitions on three different scanners in such a short time frame required significant time and effort. The authors would also like to thank Julien Cohen-Adad of Polytechnique Montréal for his great work in leading the international effort that defined most of the T1-w and T2*−w protocols used in the study, as well as Antonella Castellano, Letterio Salvatore Politi and Andrea Falini of San Raffaele Institute (Milan, Italy), who were fundamental in optimizing the PSIR protocol for Philips scanners. Additionally, the authors would like to express their gratitude for the indispensable support received from Siemens (Gerhard Laub, Sinyeob Ahn, Kevin J. Johnson), Philips (Marcello Cadioli) and General Electric (Suchandrima Banerjee, Patrick D. Koon). Finally, the authors would like to thank William A. Stern, and all the MRI technologists and staff members who with their professionalism, kindness and availability made performing this study possible. This project was supported by the Research Evaluation & Allocation Committee (REAC), School of Medicine, University of California, San Francisco.

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