Grey versus white matter segmentation of the human conus medullaris: reliability and variability in healthy volunteers

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ABSTRACT

Background and Purpose: Magnetic resonance imaging (MRI) derived spinal cord (SC) grey and white matter (GM/WM) volume are useful indirect measures of atrophy and neurodegeneration over time, typically obtained in the upper SC. Neuropathological evidence suggests that in certain neurological conditions, early degeneration may occur as low as the sacral SC. In this study, the feasibility of GM/WM segmentation of the conus medullaris (CM) was assessed *in vivo*. **Methods:** 23 healthy volunteers (11 female, mean age 47 years) underwent high-resolution 3T MRI of the CM using a 3D fast field echo sequence. Reproducibility of the volume measurements was assessed in 5 subjects (2 female, 25-37 years) by one rater who repeated the analysis 3 times and also with 2 additional raters working independently in order to calculate the intra- and inter-rater coefficient of variation (COV), respectively. Furthermore, the influence of age, gender, spine and SC metrics on tissue-specific measures of the CM was investigated. **Results:** Volumetric CM analyses (N=23) for the SC, GM, and WM revealed a mean (SD) total volume of CM-TV = 1746.9 (296.7) mm^3, CM-GM-TV = 731.2 (106.0) mm^3, and CM-WM-TV = 1014.6 (211.3) mm^3, respectively. The intra-rater COV for measuring the CM-TV and CM-GM-TV was 3.38% and 7.42%, respectively; the inter-rater COV was 3.43% and 10.80%, respectively. Using age, gender, spine and SC metrics in regression models substantially reduced group variability for CM-TV, CM-WM-TV and CM-GM-TV by up to 39.2%, 42.7%, and 21.2%, respectively. **Conclusions:** The results from this study demonstrate the feasibility of obtaining tissue-specific volume measurements in the CM by means of MRI with good reproducibility and provide normative data for future applications in neurological diseases affecting the lower SC.

INTRODUCTION

Spinal cord (SC) grey matter (GM) and white matter (WM) cross-sectional area (CSA) or volume measures obtained by means of magnetic resonance imaging (MRI) are useful for indirectly assessing the degree of atrophy (i.e. axonal loss) and degeneration over time in neurological disease.[1] In multiple sclerosis (MS), these measures in the cervical and thoracic SC have been shown to correlate independently with measures of physical disability.[2-4] Moreover, it has recently been demonstrated in healthy controls that the sensitivity and specificity of these morphometric assessments could improve further by accounting for the effects of different covariates such as age, gender, brain volumes and skull- and vertebra-derived metrics.[5]

A number of clinically feasible MRI acquisition protocols have been proposed recently, which allow the clear distinction between GM and WM in the SC.[6-8] However, their use is currently restricted to the mid- and upper SC due to technical challenges associated with imaging lower levels of the SC. For instance, due to the anatomical location of the lower SC, longer examination times are often required in order to achieve sufficient coverage and signal-to-noise ratio (SNR) for high-resolution imaging. On the other hand, in the cervical SC, the receiving elements of the radiofrequency (RF) coil can be positioned closer to the region of interest, achieving higher SNR and thus reducing the total examination time; shorter examination times are important to minimize image artifacts due to involuntary subject motion. Furthermore, lower SC imaging can be significantly affected by flow artifacts from major blood vessels in close proximity to the investigated tissue and motion artifacts from bowel peristalsis and respiration.

Using healthy volunteers, the feasibility of obtaining tissue-specific (i.e. GM and WM) measures in the lower SC has recently been demonstrated,[9] opening up possibilities to investigate the neural basis for lower urinary tract (LUT) and sexual dysfunction, which are commonly reported following neurological diseases such as spinal cord injury (SCI),[10,11] MS[12,13] and multiple system atrophy (MSA).[14,15] Given the positional variation of the lower SC relative to the spine, the identification of the lumbosacral enlargement (LSE) has been suggested as an intrinsic imaging biomarker for alignment and assessments of neurological segments relevant for the functioning of the lower limbs and the LUT. Notwithstanding the value and potential clinical utility of this method, it is possible that tissue damage can also be present below the level of the LSE extending to the conus medullaris (CM),[16] and such changes may not be fully characterised by studying the LSE alone. However, imaging methods have yet to be established to study the CM and overcoming the technical challenges due to the diminishing size of this structure when moving caudally along the spine.

In this work, the feasibility of tissue-specific segmentation of the CM is assessed *in vivo* using a clinical 3T MRI system in a cohort of healthy volunteers in order to provide a first report of normative tissue-specific morphometric characteristics of the CM. In addition, the source of inter-subject variability and the influence of age, gender, spine and SC metrics on tissue-specific measures of the CM are investigated to guide future assessments of neurological diseases.

METHODS

Study Participants

Thirty-four healthy subjects with no known neurological disorder were recruited for this study. The work was approved by the local ethics committee and written informed consent was obtained from all study participants.

Magnetic Resonance Imaging

Using a 3T Philips Achieva system with RF dual-transmit technology (Philips Healthcare, Best, Netherlands) and the manufacturer's product 16-channel neurovascular (NV-16) and 15-channel SENSE receive-only RF spine coils, a conventional T2-weighted image of the thoracolumbar spine was first obtained in the sagittal plane using a turbo spin-echo (TSE) sequence and was used to facilitate prescription of subsequent scans perpendicular to the longitudinal axis of the cord. The imaging parameters for the T2-weighted TSE were: TR = 3575 ms, TE = 100 ms, flip angle $\alpha = 90°$, FOV = $300 \times 180\ mm^2$, voxel size = $0.8 \times 0.8 \times 3\ mm^3$, NEX = 2, slices = 15, acquisition time 3:48 min.

A 3D slab-selective fast field-echo (3D-FFE) sequence with fat suppression was acquired in the axial-oblique plane (i.e. slices perpendicular to the longitudinal axis of the cord). The outer slice of the imaging volume was positioned at the superior margin of the T11 vertebral body with the volume extending caudally towards the inferior margin of the L1 vertebral body covering the LSE and the CM (Figure 1A).[9] The following parameters were used:

repetition time = 23 ms, echo time = 4.4 ms, flip angle $\alpha = 10°$, field of view = 180 × 180 mm^2, voxel size = 0.5 × 0.5 × 5 mm^3, number of averages = 8, 19 slices and scanning time of 19:27 min.

In order to minimise motion artefacts, the torso was restrained using velcro straps. Hip flexion was achieved through the use of a large foam wedge that increased the level of contact between the lower back and the coil surface.[9] Every effort was made to ensure the participants underwent a comfortable examination.

Image analysis

For each slice, tissue-specific image segmentation was performed manually in all cases using JIM 6.0 (http://www.xinapse.com) providing corresponding CSA measurements for the cord and GM. The slice with the largest cord CSA between T11-L1 vertebral bodies was identified for each subject as the LSE slice,[9] and all slices were subsequently renumbered according to their distance from the LSE slice (hereinafter referred to as 'slice 0', see Figure 1B). The CM was identified for each subject independently of the others, hence the CM number of slices (CM-n-slices) was subject-specific. In order to facilitate interpretation of the results obtained in the CM segment, corresponding measurements were also obtained for the LSE region as previously described.[9] Briefly, total volume (TV) measures were calculated for a 15mm segment around the LSE (LSE-TV, LSE-GM-TV) from 3 adjacent slices (+1/0/-1) and for the CM (CM-TV, CM-GM-TV) from a variable CM-n-slices (0, -1, -2…/-n) down to the tip of the CM; the tip of the CM was defined as the last axial slice on which the cord could still be visible, with the two preceding slices showing the cord with a larger diameter (superiorly)

and no cord at all (inferiorly). Corresponding WM measures were calculated on an individual basis as the difference between pairwise SC and GM measures.

Reproducibility assessment

Images from 5 healthy volunteers (2 female, mean age 30 years; range 24.5-37.1 years) were analysed 3 times by 3 raters independently with a minimum of 2 weeks gap in-between each rating, in order to assess the reproducibility of all measures. For each subject, the position of the LSE (slice 0) was determined based on the median position of the largest CSA between T11-L1 across all ratings for the same subject.

Given the inter-subject biological variability in length of the CM, intra- and inter-rater slice ranges were calculated for CM-n-slices. Additional slice-wise analyses were conducted for slices +1/0/-1/…/-6, which was the most appropriate range of slices to account for the variability in length of the CM in the 5 volunteers.

Normalisation covariates

Normalisation metrics such as the thecal-sac cross-sectional area at T12 vertebral level (TS_CSA_T12), the spine length from C2-L5 vertebrae (Length_C2_L5), cord length from the LSE to the tip of the CM (Length_LSE_CMtip), cord length from C2 level to the tip of CM (Length_C2_CMtip), cord length from C2 level to the LSE (Length_C2_LSE), T12 vertebra anterior height (T12_AH), T12 vertebra central height (T12_CH), T12 vertebra posterior height (T12_PH) and T12 vertebra anteroposterior length (T12_AP) were obtained

from the high resolution T2-weighted acquisition and from a two-station scout image of the entire spine, which was subsequently reconstructed without overlap (Figure 2).

Statistical Analysis

Statistical analysis was performed using SPSS 24.0 (SPSS, Chicago, Ill., USA). Results are typically reported as mean and standard deviation (SD). For the assessment of reproducibility, the coefficient of variation (COV) was calculated using the mean and SD from the repeated measures and the equation COV= [SD/mean] × 100%. In order to estimate the intra- and inter-rater measurement error relative to the biological variability between subjects, intra-class correlation coefficient (ICC) estimates and their 95% confident intervals were calculated based on single measurements, 2-way mixed-effects model with absolute-agreement/consistency for intra-rater/inter-rater estimates, respectively.

Gender effects were analysed using two-tailed paired student's t-tests. For tissue-specific volume measures, gender effects were also explored using analysis of covariance. In addition, multiple regression analysis was used to further explore the effect of population descriptives i.e. age, gender, weight and body height. Using the Pearson's correlation coefficient, all spine and SC metrics having a coefficient greater than 0.45, with at least two of the three CM volume measures, were selected as candidates for subsequent normalisation using a previously described regression-based residual method:[5]

$$V^S_{normalised} = V^S_{measured} + a\,(X_{mean} - X^S_{measured}) + b\,(Y_{mean} - Y^S_{measured}) + ..$$

where $V^S_{normalised}$ is the normalised CM volume measured in a given subject (*S*), $V^S_{measured}$ is the measured CM volume in the same subject, $X_{mean}, Y_{mean}, ..$ are the average spine/spinal cord metrics over all subjects, $X^S_{measured}, Y^S_{measured},$ are the measured values in subject *S*, and $a, b, ..$ the regression coefficients derived from the multi-linear fit.

For the best regression analysis models comprising significantly contributing variables in explaining the CM outcome, the effect of normalisation was assessed by comparing the COV before and after normalisation; COV, in this case, was the ratio of the group standard SD and mean calculated separately for each CM tissue volume. Additional combinations with age and/or gender were investigated irrespective of their explanatory value to the respective model.

RESULTS

After exclusion of datasets with imaging artefacts (N=1), degenerative disc changes (N=2), additional vertebrae (N=2), and/or CM tip not included in the imaging volume (N=6), 23 subjects (11 female, mean age 47 years; range 25-73 years) were considered for normative CM analyses.

Volumetric CM analyses for the SC, GM, and WM revealed a mean (SD) total volume of CM-TV = 1746.9 (296.7) mm^3, CM-GM-TV = 731.2 (106.0) mm^3, and CM-WM-TV = 1014.6 (211.3) mm^3, respectively.

Table 1 summarises the results of the reproducibility assessment for SC volumes (i.e. LSE and CM) from five healthy volunteers (25-37 years old, 2 female). Slice-wise results are provided for the CSA of the cord (Table 2) and GM (Table 3), separately. The COV of the tissue-specific morphometric measures increased considerably when moving caudally from the LSE slice towards the tip of the CM. In addition, inter-rater results were worse than intra-rater results in most cases, and the worst COV values calculated as a whole were from inter-rater evaluations of GM-CSA.

In general, considering population descriptives (N=23), males had larger spine and spinal cord metrics as well as CM volumes than females, with significantly larger CM-WM-TV also when adjusted for age (separate exploratory analyses). Table 4 summarises the spine/SC metrics, LSE and CM measures by gender.

Figure 3 shows plots of group mean CSA area for each tissue-type spanning the LSE down to the CM; notably, the group mean GM-CSA and WM-CSA did not vary proportionally to the group mean cord CSA caudally from the level of the LSE, with GM-CSA at its largest two slices below the LSE slice. Figure 4 shows scatterplots of LSE and CM volume measures as a function of age in males and females.

Table 5 shows results from correlations between the CM volume measures and the spine and SC metrics, showing that Length_LSE_CMtip correlated the highest with all the individual CM volume measures. T12_PH, Length_C2_LSE and Length_C2_CMtip also showed high correlations with two out of the three CM volume measures. The metrics with the highest correlation coefficients shown on the table were used in a subsequent multiple regression analysis.

The best regression models were found for combinations with the predictor variables Length_LSE_CMtip and T12_PH. Additional variable age and/or gender did not show significant contributions in explaining the CM volumes despite occasional minor improvements in the performance of the respective models. Table 6 shows the COV reductions per CM tissue-type for the best regression models (i.e. combination of spine and SC metrics) with the highest adjusted R^2 value. Models based on a single variable (i.e. T12_PH, Length_C2_CMtip, Length_C2_L5, Length_C2_LSE) performed rather poorly, among which Length_LSE_CMtip achieved the best performance. For the best models normalisation formulas reduced the COV of measured data for CM-TV from 16.98% to as low as 10.32%, for CM-WM-TV from 20.83% to 11.94% and for CM-GM-TV from 14.50% to 11.42%.

DISCUSSION

In this study we demonstrated the feasibility of obtaining tissue-specific volume measures of the CM and provided a first report on normative values from a cohort of healthy volunteers. Additionally, we examined the influence of population descriptives, spine and SC metrics on CM tissue-specific volume measures and identified normalisation strategies that can reduce the variability of these measures, making their clinical adoption feasible to study neurological diseases known to affect the lower SC.[10-15] Overall the proposed imaging protocol was well tolerated by all subjects, consistent with the original study of the LSE.[9]

The CM has considerable importance in the neurological control of somatic lower limb and pelvic floor functions, as well as control of autonomic LUT, lower bowel and sexual functions.[17] Due to the technical challenges associated with imaging the lower SC, the identification of the LSE has recently been suggested as an intrinsic imaging biomarker for alignment and assessments of neurological segments relevant for the functioning of the lower limbs and the LUT.[9] However, neuropathological evidence suggests that in certain conditions early degeneration may occur below the level of the LSE.[16] With the appropriate methodological considerations we managed in this study to overcome the technical challenges associated with the diminishing size of the CM moving caudally from the LSE to present a reliable method for tissue-specific volume measurements, which may find immediate clinical utility given its potential relevance.

The reproducibility results for the LSE segment in this study were found to be in agreement with the previously reported findings, confirming the robustness of the segmentation method.

Specifically, in this study the intra- and inter-rater COV values were 2.70% and 2.33% compared to 2% and 2.5% in the original report for the LSE-TV, 7.32% and 9.32% compared to 8% and 8.6% for the LSE-GM-TV.[9] The reproducibility of the measures obtained in the CM are marginally lower compared to the LSE, which is consistent with the variable and smaller diameter of this structure, confirmed by the slice-wise analyses. Future work will aim to improve the image acquisition protocol further i.e. to achieve higher resolution, but also to explore the potential of using fully automated segmentation methods, previously shown to be suitable for the upper SC.[18]

Tissue-specific volume measures in the CM spanned a variable number of slices (CM-n-slices), and this is consistent with the expected biological inter-subject variability. Normative data from tissue-specific morphometric assessments of the CM are not currently available in the literature for direct comparison. The results from this study showed that males generally had higher CM volumes than females, with significantly higher CM-WM-TV also when adjusted for age. In addition, the results from the multiple regression analysis showed that SC and spine metrics combined with age contributed the greatest in explaining the variance in CM volumes. These results are consistent with the findings from previous studies evaluating the CSA of the cervical cord only,[19,20] or tissue-specific CSA measures obtained both at the cervical and thoracic cord levels.[4]

In this study, possible normalisation strategies were explored based on a number of spine and SC metrics. Among the metrics tested, Length_LSE_CMtip was found to be highly correlated with all CM tissue-specific volume measures. This was expected considering the formula to calculate a cone volume, which is proportional to its height. T12_PH, Length_C2_L5,

Length_C2_LSE and Length_C2_CMtip also showed significant correlations with two out of the three CM volume measures. While it is entirely possible that other normalisation metrics such as brain GM, brain WM, total brain volumes and total intracranial volume may also provide significant correlations with CM volume measures,[19,21] this study focussed mostly on metrics that are less likely to be affected by neurological disease and are obtainable from routine SC investigations, whereby data from brain imaging may not always be available. Notwithstanding, future studies are needed to investigate further normalisation metrics, particularly the total intracranial volume, in order to explain and reduce the variability for SC volume assessments.

Using the proposed spine and SC metrics, age and gender to normalise CM volume measures based on a previously described regression-based residual method, it has been shown that the COV of measured data for CM-TV, CM-WM-TV and CM-GM-TV can be reduced by up to 39.2%, 42.7% and 21.2%, respectively. These results can have profound implications for future investigations of neurological disease affecting the lower SC. For instance, without normalisation, to detect a 10% difference between patients and controls with 80% power, at 5% significance, would require 94 subjects (47 patients, 47 controls) for CM-TV, 137 subjects for CM-WM-TV and 67 subjects for CM-GM-TV; after normalisation, the samples sizes for CM-TV, CM-WM-TV and CM-GM-TV required would be 29, 40 and 40, respectively.

The main limitation of the present study included the length of the examination time required to obtain coverage of the spine and spinal cord anatomy. A further limitation was that the entire image analysis was performed manually, although this was shown to be reproducible in

this study. Finally, investigation of additional normalisation metrics could provide further correlations with the CM volume measures. Technically, future work will need to address these points and in particular the possibility of reducing scan time in order to ensure additional patient comfort. Recent developments in commercially available hardware and software make it possible to image the entire spine without the need to reposition the subject in the scanner, offer improved SNR performance through digital broadband MR architecture, while at the same time offering faster imaging options for enhanced workflow. Such time saving could be exploited also to extend the imaging protocol to acquire data to reconstruct biophysically meaningful features linked for example to myelin content or microstructural properties. In parallel, automatic methods for CM segmentation should be explored together with potential further normalisation methods.

Ultimately, we aim to translate the method presented here to clinical studies of neurological diseases affecting the lower SC, such as the study of demyelinating plaques in MS and neurodegenerative changes affecting different neuronal populations in MSA.[16] Devising a more comprehensive imaging protocol to include quantitative MR acquisition methods would be of great value in disease states to further our understanding of the underlying pathophysiological processes affecting the CM *in vivo*.

In summary, obtaining tissue-specific CM volume measures *in vivo* using a clinical 3T system is possible and the presented spine and SC metrics should be used in future studies of neurological disease while working on improving further methods to address the reported inter-subject variability, hence improve the sensitivity and statistical power of the CM morphometric assessments.

REFERENCES

1. Wheeler-Kingshott CA, Stroman PW, Schwab JM et al. The current state-of-the-art of spinal cord imaging: applications. Neuroimage 2014;84:1082-93.
2. Losseff NA, Webb SL, O'Riordan JI et al. Spinal cord atrophy and disability in multiple sclerosis. A new reproducible and sensitive MRI method with potential to monitor disease progression. Brain 1996;119:701-8.
3. Schlaeger R, Papinutto N, Panara V et al. Spinal cord gray matter atrophy correlates with multiple sclerosis disability. Ann Neurol 2014;76:568-80.
4. Schlaeger R, Papinutto N, Zhu AH et al. Association between thoracic spinal cord gray matter atrophy and disability in multiple sclerosis. JAMA Neurol 2015;72:897-904.
5. Papinutto N, Schlaeger R, Panara V et al. Age, gender and normalization covariates for spinal cord gray matter and total cross-sectional areas at cervical and thoracic levels: A 2D phase sensitive inversion recovery imaging study. PloS One 2015;10:e0118576.
6. Yiannakas MC, Kearney H, Samson RS et al. Feasibility of grey matter and white matter segmentation of the upper cervical cord in vivo: A pilot study with application to magnetisation transfer measurements. Neuroimage 2012;63:1054-9.
7. Kearney H, Yiannakas MC, Abdel-Aziz K et al. Improved MRI quantification of spinal cord atrophy in multiple sclerosis. J Magn Reson Imaging 2014;39:617-23.
8. Papinutto N, Schlaeger R, Panara V et al. 2D phase-sensitive inversion recovery imaging to measure in vivo spinal cord gray and white matter areas in clinically feasible acquisition times. J Magn Reson Imaging 2015;42:698-708.

9. Yiannakas MC, Kakar P, Hoy LR, Miler DH, Wheeler-Kingshott CA. The use of the lumbosacral enlargement as an intrinsic imaging biomarker: feasibility of grey matter and white matter cross-sectional area measurements using MRI at 3T. PLoS One 2014;9:e105544.

10. Anderson KD, Borisoff JF, Johnson RD, Stiens SA, Elliott SL. The impact of spinal cord injury on sexual function: concerns of the general population. Spinal Cord 2007;45:328-37.

11. Watanabe T, Vaccaro AR, Kumon H, Welch WC, Rivas DA, Chancellor MB. High incidence of occult neurogenic bladder dysfunction in neurologically intact patients with thoracolumbar spinal injuries. J Urol 1998;159:965-8.

12. McCombe PA, Gordon TP, Jackson MW. Bladder dysfunction in multiple sclerosis. Expert Rev Neurother 2009;9:331-40.

13. Fernandez O. Mechanisms and current treatments of urogenital dysfunction in multiple sclerosis. J Neurol 2002;249:1-8.

14. Sakakibara R, Hattori T, Tojo M, Yamanishi T, Yasuda K, Hirayama K. Micturitional disturbance in multiple system atrophy. Jpn J Psychiatry Neurol 1993;47:591-8.

15. Sakakibara R, Hattori T, Uchiyama T et al. Urinary dysfunction and orthostatic hypotension in multiple system atrophy: which is the more common and earlier manifestation? J Neurol Neurosurg Psychiatry 2000;68:65-9.

16. Konno H, Yamamoto T, Iwasaki, Iizuka H. Shy-Drager syndrome and amyotrophic lateral sclerosis. Cytoarchitectonic and morphometric studies of sacral autonomic neurons. J Neurol Sci 1986;73:193-204.

17. Panicker JN, Fowler CJ, Kessler TM. Lower urinary tract dysfunction in the neurological patient: clinical assessment and management. Lancet Neurol 2015;14:720-32.

18. Prados F, Ashburner J, Blaiotta C et al. Spinal cord grey matter segmentation challenge. Neuroimage 2017;152:312-29.

19. Mann RS, Constantinescu CS, Tench CR. Upper cervical spinal cord cross-sectional area in relapsing remitting multiple sclerosis: application of a new technique for measuring cross-sectional area on magnetic resonance images. J Magn Reson Imaging 2007;26:61-5.

20. Ishikawa M, Matsumoto M, Fujimura Y, Chiba K, Toyama Y. Changes of cervical spinal cord and cervical spinal canal with age in asymptomatic subjects. Spinal Cord. 2003;41:159-63.

21. Rashid W, Davies GR, Chard DT et al. Upper cervical cord area in early relapsing-remitting multiple sclerosis: cross-sectional study of factors influencing cord size. J Magn Reson Imaging 2006;23:473-6.

TABLES

Table 1. Results from the reproducibility assessment (N=5).

Measure	Mean (SD) [mm^3]	Intra-rater COV [%]	Inter-rater COV [%]	Intra-rater ICC [95% CI]	Inter-rater ICC [95% CI]
LSE-TV	938.6 (97.5)	2.70	2.33	0.91 [0.75, 0.97]	0.93 [0.84, 0.97]
LSE-GM-TV	348.0 (48.0)	7.32	9.32	0.60 [0.31, 0.82]	0.53 [0.22, 0.79]
CM-TV	1575.3 (359.4)	3.38	3.43	0.97 [0.92, 0.99]	0.98 [0.94, 0.99]
CM-GM-TV	620.4 (146.5)	7.42	10.80	0.86 [0.71, 0.95]	0.86 [0.71, 0.95]
CM-n-Slices Median (Range)	10 (7-12)	5.52	4.31	0.77 [0.51, 0.91]	0.85 [0.69, 0.94]

LSE-TV: Total lumbosacral enlargement volume (in mm^3); LSE-GM-TV: Total grey matter volume in LSE; CM-TV: Total conus medullaris volume; CM-GM-TV: Total grey matter volume in CM; CM-n-Slices: Number of slices spanning the conus medullaris; COV: Coefficient of variation; ICC: Intra-class correlation coefficient, indicating the level of reliability; CI: Confidence interval; SD: Standard deviation.

Table 2. Slice-wise reproducibility assessment results for cord cross-sectional area (N=5).

Slice number	CSA [mm^2] Mean (SD)	Intra-rater COV [%]	Inter-rater COV [%]	Intra-rater ICC [95% CI]	Inter-rater ICC [95% CI]
+1	62.01 (5.09)	2.86	2.61	0.83 [0.62, 0.94]	0.86 [0.70, 0.94]
0	63.75 (6.96)	3.52	2.99	0.86 [0.65, 0.95]	0.89 [0.76, 0.96]
-1	61.96 (7.8)	2.69	2.62	0.94 [0.85, 0.98]	0.95 [0.88, 0.98]
-2	56.68 (10.22)	3.07	2.75	0.96 [0.90, 0.99]	0.97 [0.93, 0.99]
-3	44.54 (12.34)	4.97	5.67	0.96 [0.89, 0.98]	0.96 [0.90, 0.98]
-4	33.59 (13.06)	4.54	6.94	0.98 [0.96, 0.99]	0.97 [0.93, 0.99]
-5	22.79 (11.15)	6.02	7.75	0.98 [0.95, 0.99]	0.97 [0.94, 0.99]
-6	14.66 (7.84)	5.65	7.59	0.98 [0.96, 0.99]	0.99 [0.96, 0.99]

CSA: Cross-sectional area for the spinal cord in mm^2; COV: Coefficient of variation; ICC: Intra-class correlation coefficient, indicating the level of reliability; CI: Confidence interval; SD: Standard deviation.

Table 3. Slice-wise reproducibility assessment results for grey matter cross-sectional area (N=5).

Slice number	GM-CSA [mm^2] Mean (SD)	Intra-rater COV [%]	Inter-rater COV [%]	Intra-rater ICC [95% CI]	Inter-rater ICC [95% CI]
1	21.33 (2.92)	8.34	9.81	0.47 [0.16, 0.75]	0.40 [0.08, 0.71]
0	23.70 (3.39)	7.74	9.85	0.59 [0.30, 0.82]	0.49 [0.18, 0.76]
-1	24.58 (3.67)	7.51	9.60	0.64 [0.37, 0.85]	0.59 [0.29, 0.82]
-2	23.70 (4.74)	7.23	10.09	0.82 [0.63, 0.93]	0.81 [0.61, 0.92]
-3	19.47 (5.77)	7.96	11.42	0.90 [0.78, 0.96]	0.87 [0.71, 0.95]
-4	14.08 (6.31)	9.20	12.91	0.94 [0.87, 0.98]	0.95 [0.88, 0.98]
-5	8.76 (5.23)	14.20	18.63	0.92 [0.82, 0.97]	0.92 [0.82, 0.97]
-6	5.53 (3.88)	13.02	18.48	0.94 [0.87, 0.98]	0.94 [0.85, 0.98]

GM-CSA: Grey matter cross-sectional area in mm^2; COV: Coefficient of variation; ICC: Intra-class correlation coefficient; CI: Confidence interval; SD: Standard deviation.

Table 4. Group characteristics with spine/spinal cord metrics, LSE and conus medullaris measures by gender (N=23) (values not adjusted).

	Female (N=11)				**Male** (N=12)				t-test
	Mean	(SD)	Minimum	Maximum	Mean	(SD)	Minimum	Maximum	p value
Age	47.0	(18.3)	25.9	73.6	47.7	(10.6)	33.6	62.2	n.s.
Spine/spinal cord metrics [mm]									
TS_CSA_T12	236.6	(39.0)	164.8	303.1	230.3	(48.5)	167.0	332.0	n.s.
T12_AH	23.3	(1.4)	21.3	25.7	24.0	(1.6)	20.6	25.6	n.s.
T12_AP	25.9	(1.9)	23.2	29.7	29.8	(2.8)	24.7	35.8	0.001**
T12_CH	22.0	(1.5)	18.4	23.8	23.6	(2.2)	19.6	27.2	0.058°
T12_PH	24.4	(1.4)	22.5	27.3	26.5	(1.7)	23.1	28.3	0.004**
Length_LSE_CMtip	42.0	(6.0)	30.7	53.4	49.0	(7.4)	36.5	59.6	0.022*
Length_C2_CMtip	397.2	(14.8)	370.1	418.6	435.5	(19.8)	393.7	477.5	<0.001***
Length_C2_LSE	355.2	(12.0)	334.6	374.0	386.5	(16.3)	357.2	423.3	<0.001***
Length_C2_L5	542.5	(15.3)	513.8	561.6	594.8	(28.1)	548.5	653.9	<0.001***
LSE volumes [mm^3]									
LSE-TV	885.4	(100.2)	721.4	1089.0	921.1	(87.3)	804.9	1142.8	n.s.
LSE-GM-TV	364.0	(57.0)	272.6	472.1	349.8	(31.5)	303.6	397.2	n.s.
LSE-WM-TV	521.5	(57.2)	448.8	616.9	571.3	(85.9)	407.7	751.9	n.s.
Conus medullaris volumes [mm^3]									
CM-TV	1628.1	(221.3)	1335.6	1989.3	1855.8	(323.2)	1173.8	2301.2	0.064°
CM-GM-TV	704.8	(115.1)	527.8	849.9	755.3	(95.3)	579.7	909.9	n.s.
CM-WM-TV	924.2	(136.2)	769.1	1200.1	1097.5	(238.3)	594.0	1450.9	0.047*

CM = Conus medullaris; LSE = Lumbosacral enlargement; TS_CSA_T12 = Thecal-sac cross-sectional area at T12 vertebra; Length_C2_L5 = Spine length from C2 to L5; Length_LSE_CMtip = Cord length from the LSE to the tip of the CM; Length_C2_CMtip = Cord length from C2 to the tip of CM; Length_C2_LSE = Cord length from C2 to the LSE; T12_AH = T12 anterior height; T12_CH = T12 central height; T12_PH = T12 posterior height; T12_AP = T12 anteroposterior length; LSE-TV = Total volume of the LSE; LSE-GM-TV = Total grey matter volume of the LSE; LSE-WM-TV = Total white matter volume of the LSE; CM-TV = Total volume of the CM; CM-GM-TV = Total grey matter volume of

the CM; CM-WM-TV = Total white matter volume of the CM; SD = Standard deviation; two-tailed paired student's t-tests, given in t-values with significance levels: ° $p<0.1$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, n.s. = not significant.

Table 5. Pearson's correlation coefficients and p values (brackets) between the volume measures and other spine and spinal cord metrics (N=23).

Measure	TS_CSA_T12	T12_AH	T12_AP	T12_CH	T12_PH	Length_LSE_CMtip	Length_C2_CMtip	Length_C2_LSE	Length_C2_L5
CM-TV	0.14	0.24	0.35	0.39°	0.58**	0.70***	0.55**	0.43*	0.52*
	(0.95)	(0.27)	(0.11)	(0.07)	(0.004)	(<0.001)	(0.006)	(0.04)	(0.01)
CM-GM-TV	-0.23	0.02	0.16	0.23	0.30	0.58**	0.39°	0.28	0.35
	(0.29)	(0.94)	(0.48)	(0.29)	(0.16)	(0.004)	(0.06)	(0.20)	(0.10)
CM-WM-TV	0.13	0.33	0.40°	0.43*	0.66**	0.69***	0.58**	0.46*	0.55**
	(0.55)	(0.13)	(0.06)	(0.04)	(0.001)	(<0.001)	(0.004)	(0.03)	(0.006)

CM = Conus medullaris; CM-TV = Total volume of the CM; CM-GM-TV = Total grey matter volume of the CM; CM-WM-TV = Total white matter volume of the CM; TS_CSA_T12 = Thecal-sac cross-sectional area at T12 vertebra; Length_C2_L5 = Spine length from C2 to L5; Length_LSE_CMtip = Cord length from the LSE to the tip of the CM; Length_C2_CMtip = Cord length from C2 to the tip of CM; Length_C2_LSE = Cord length from C2 to the LSE; T12_AH = T12 anterior height; T12_CH = T12 central height; T12_PH = T12 posterior height; T12_AP = T12 anteroposterior length; statistical significance levels: ° p<0.1, * p<0.05, ** p<0.01, *** p<0.001.

Table 6. Measured and calculated coefficient of variation by conus medullaris tissue-type for best regression models.

	Model	Age	Gender	T12_PH	Length_ LSE_CMtip	Adj. R^2	%COV (Calculated)	%COV (Measured)
CM-TV	1	-	-	-	27.67 *	0.47	12.13	16.98
	2	-	-	54.35 *	21.80 *	0.54	10.99	
	2b	-4.84	-	62.90 *	19.26 *	0.58	10.32	
	2c	-4.79	61.26	70.64 *	20.42 *	0.56	10.17	
CM-WM-TV	1	-	-	-	19.48 *	0.45	15.06	20.83
	2	-	-	50.00 *	14.08 *	0.60	12.65	
	2b	-3.11	-	55.49 *	12.44 *	0.62	11.94	
	2c	-3.08	48.15	61.58 *	13.54 *	0.61	11.73	
CM-GM-TV	1	-	-	-	8.20 *	0.30	11.79	14.50
	1b	-1.54	-	-	7.68 *	0.32	11.42	
	1c	-1.54	2.88	-	7.77 *	0.28	11.41	
	2	-	-	7.83	3.42 *	0.27	11.78	

CM = Conus medullaris; CM-TV = Total volume of the CM; CM-GM-TV = Total grey matter volume of the CM; CM-WM-TV = Total white matter volume of the CM; Length_LSE_CMtip = Cord length from the LSE to the tip of the CM; Length_C2_LSE = Cord length from C2 to the LSE; T12_AH = T12 anterior height; %COV = coefficient of variation in percentages; * variable significantly contributing to the respective model.

FIGURES

Figure 1.

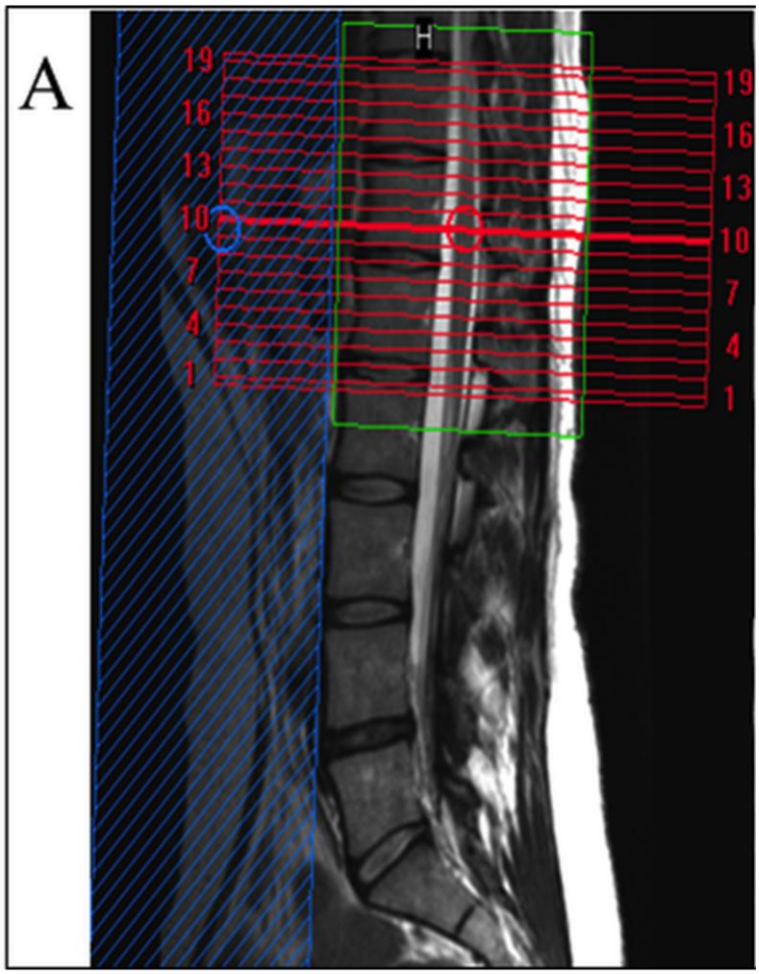

Figure 1. A) Imaging the lumbosacral spinal cord in the axial plane with the imaging volume positioned at the superior margin of T11 vertebral body and extending caudally to cover the lumbosacral enlargement and the conus medullaris; B) Midline sagittal and 15 out of 19 axial images (voxel size = 0.5x0.5x5 mm^3) shown in a healthy volunteer. For display purposes, cord and grey matter segmentations are shown on alternating slices. In each case, the slice with the largest cross-sectional area between T11-L1 (lumbosacral enlargement; LSE) was identified (slice 0), and all the remaining consecutive slices moving caudally from the LSE slice towards the tip of the conus medullaris (0/-1/-2/-3…/-n) were subsequently renumbered

(Note: original slice acquisition numbers are displayed in red font and relative numbers to the LSE are displayed in white font).

Figure 1.

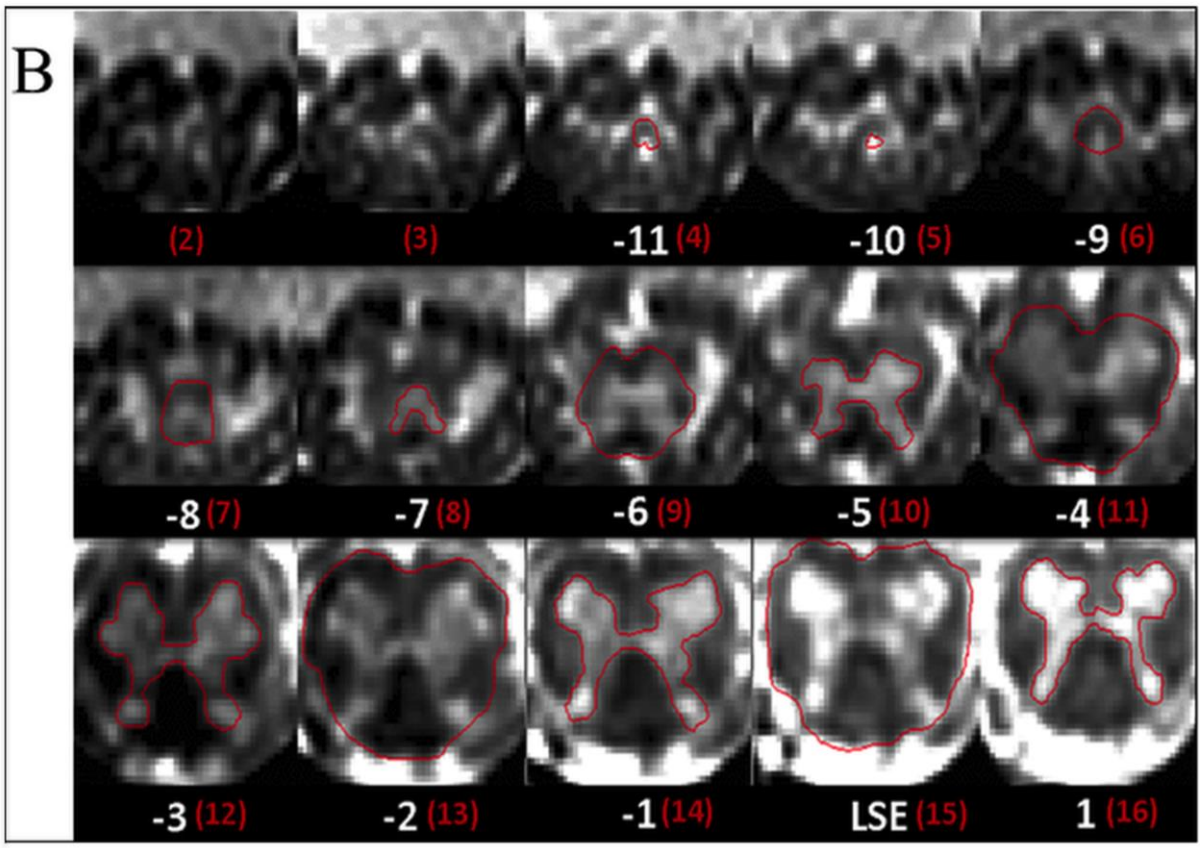

Figure 2.

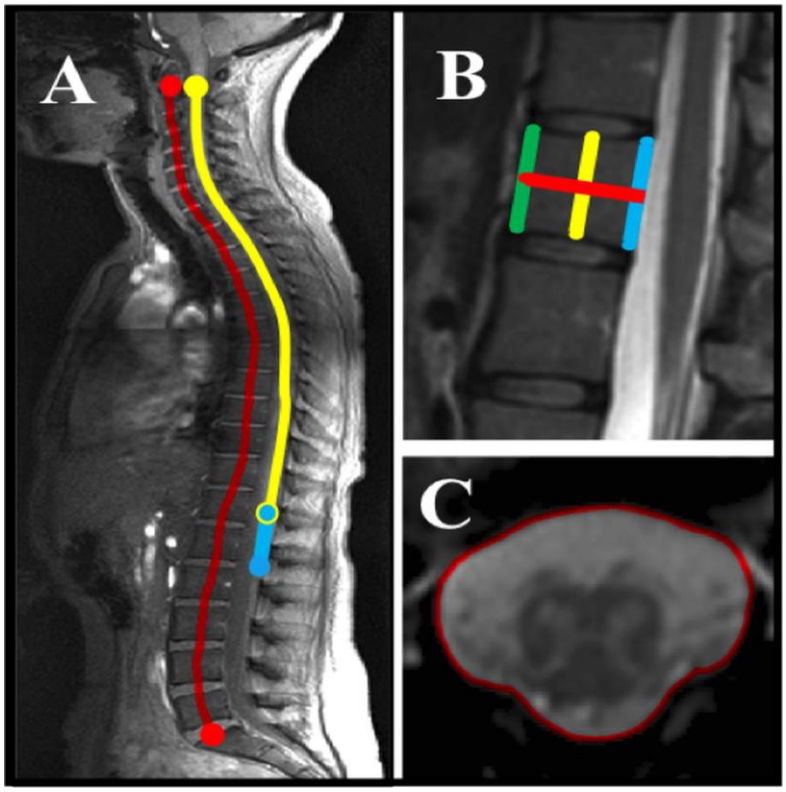

Figure 2. Spine and spinal cord normalisation metrics: A) Spine length from C2 to L5 (Length_C2_L5; red), cord length from the lumbosacral enlargement (LSE) to the tip of the conus medullaris (CM) (Length_LSE_CMtip; cyan), cord length from C2 to the LSE (Length_C2_LSE; yellow) and cord length from C2 to the tip of CM (Length_C2_CMtip; sum of yellow and cyan); B) T12 anterior height (T12_AH; green), T12 central height (T12_CH; yellow), T12 posterior height (T12_PH; cyan) and T12 anteroposterior length (T12_AP; red); C) thecal-sac cross-sectional area (TS_CSA_T12; red).

Figure 3.

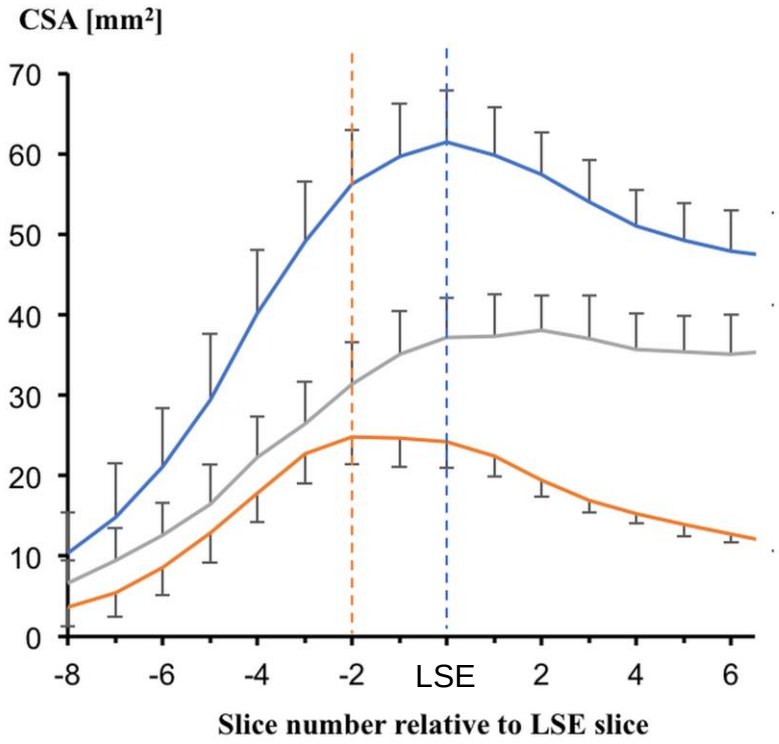

Figure 3. Plots of group mean cross-sectional area (CSA) for each tissue-type spanning the lumbosacral enlargement (LSE) down to the conus medullaris for the spinal cord (blue line), white matter (grey line), and grey matter (orange line). One-sided error bars representing one standard deviation of corresponding group means. Peak GM-CSA is indicated by the dashed orange line two slices below the LSE position.

Figure 4.

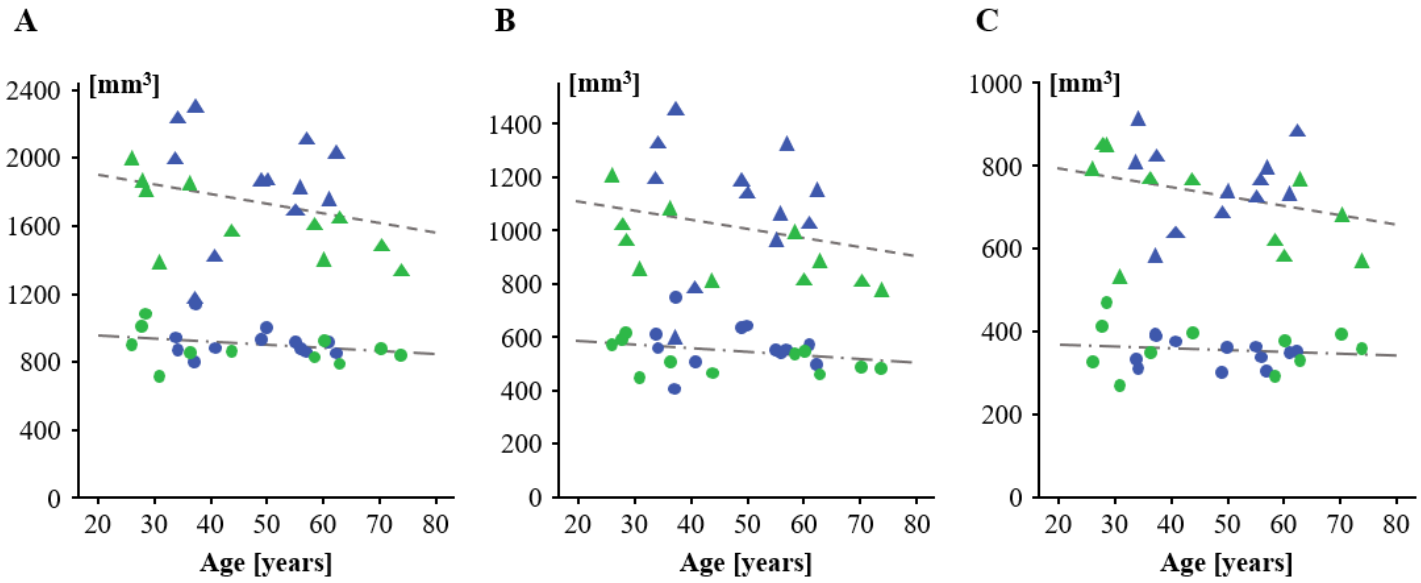

Figure 4. Relationship between volumetric outcome measures and age. A) Total volume (TV), B) white matter (WM), and C) grey matter (GM) volumes as a function of age in males (blue) and females (green) at the lumbosacral enlargement (LSE, shown as dots) and the conus medullaris (CM, shown as triangles) as a function of age in males (blue) and females (green). Linear regression lines are indicated for LSE (dashed line) and CM (dashed-dotted line). There was no significant relationship between spinal cord volume measures and age across all subjects. Only when analysed separately per gender, a significant linear relationship was found in females for CM-TV: b=-8.262, r=-0.682, $t_{[10]}$=-2.797, p=0.021 and CM-WM-TV: b=-4.978, r=-0.668, $t_{[10]}$=-2.691, p=0.025.