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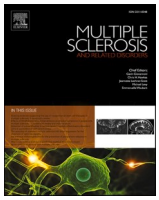

Upper cervical cord atrophy is independent of cervical cord lesion volume in early multiple sclerosis: A two-year longitudinal study

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ABSTRACT

Background: Upper cervical cord atrophy and lesions have been shown to be associated with disease and disability progression already in early relapsing-remitting multiple sclerosis (RRMS). However, their longitudinal relationship remains unclear.

Objective: To investigate the cross-sectional and longitudinal relation between focal T2 cervical cord lesion volume (CCLV) and regional and global mean upper cervical cord area (UCCA), and their relations with disability.

Methods: Over a two-year interval, subjects with RRMS ($n = 36$) and healthy controls (HC, $n = 16$) underwent annual clinical and MRI examinations. UCCA and CCLV were obtained from C1 through C4 level. Linear mixed model analysis was performed to investigate the relation between UCCA, CCLV, and disability over time.

Results: UCCA at baseline was significantly lower in RRMS subjects compared to HCs ($p = 0.003$), but did not decrease faster over time ($p \geq 0.144$). UCCA and CCLV were independent of each other at any of the time points or cervical levels, and over time. Lower baseline UCCA, but not CCLV, was related to worsening of both upper and lower extremities function over time.

Conclusion: UCCA and CCLV are independent from each other, both cross-sectionally and longitudinally, in early MS. Lower UCCA, but not CCLV, was related to increasing disability over time.

1. Introduction

Spinal cord (SC) pathology is frequently seen in multiple sclerosis (MS) and is a strong contributor to disability and disease progression (Gass et al., 2015; Moccia et al., 2019; Thompson et al., 2018a). SC pathology includes lesions, which have a major role in diagnosis and prognosis of MS (Rocca et al., 2020; Kearney et al., 2015; Thompson et al., 2018b), and tissue loss or atrophy (Biberacher et al., 2015; Brownlee et al., 2017; Losseff et al., 1996; Bot et al., 2004), which is generally assessed from upper cervical cord area (UCCA) measurements (Moccia et al., 2019; Weeda et al., 2019; Sastre-Garriga et al., 2020) and is already present in early stages of the disease (Hagstrom et al., 2017; Lukas et al., 2015).

The relation between cervical cord focal lesions and atrophy is not fully understood. The *in vivo* measurement of UCCA has shown to be robust in the presence of lesions (Weeda et al., 2019), focal lesions and

Abbreviations: 25-FWT, 25-foot walk test; 9-HPT, 9-hole peg test; AIS, Athens insomnia scale; ARR, annualized relapse rate; CCLV, cervical cord lesion volume; CIS-20, checklist individual strength; CIS, clinically isolated syndrome; CL, cervical lesion; DH, dominant hand; EDSS, expanded disability status scale; FLAIR, fluid attenuated inversion recovery; FSPGR, fast spoled gradient echo; GBSI, general boundary-shift integral; MS, multiple sclerosis; MS+CL, RRMS subject with a lesion at any cervical level; MS-CL, RRMS subject without a lesion at any cervical level; MSFC, multiple sclerosis functional composite; MSNQ, multiple sclerosis neurological screening questionnaire; MSWS, multiple sclerosis walking scale; NBV, normalized brain volume; NLV, normalized lesion volume; NDH, non-dominant hand; RRMS, relapsing-remitting multiple sclerosis; SC, spinal cord; SCT, Spinal Cord Toolbox; TE, echo time; TI, inversion time; TR, repetition time; UCCA, upper cervical cord area.

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diffuse abnormalities in the cervical cord have been found to be associated with cord atrophy (Lukas et al., 2015; Evangelou et al., 2005; Pravata et al., 2020). However, there is no longitudinal data on the relation between upper cervical cord area (UCCA) and cervical cord lesion volume (CCLV) in MS.

Therefore, we aimed to investigate the longitudinal evolution and interrelations of UCCA and CCLV in early RRMS over a two-year follow-up with annual visits. Secondly, we aimed to investigate the relation of UCCA and CCLV with physical disability.

2. Methods

2.1. Subjects

The institutional review board approved the study protocol and all participants gave written informed consent prior to participation, according to the Declaration of Helsinki.

To enable studying of the early disease course, patients included were diagnosed with clinical definite RRMS according to McDonalds 2010 criteria (Polman et al., 2011), with a maximal disease duration of 5 years and maximum expanded disability status scale (EDSS) score of 5.0. Subjects were using first-line treatment, or no treatment at all. In case of switching of treatment, MRI examinations were planned with at least 4–6 months delay (De Stefano et al., 2003). When steroids were used, MRI was delayed by 3 months (Zivadinov et al., 2013). Patients were excluded (over the course of the study) in case of (switching to) second-line treatment.

To differentiate age-related MRI changes from disease-related MRI changes, a group of age, sex and education matched healthy controls (HCs) was included. HC and MS subjects were not eligible for participation when they could not undergo MRI examination, or when they had past or current clinically relevant neurological, psychiatric or (auto) immune disorders other than MS.

Subjects visited a single-centre three times, with one-year intervals, for MRI and clinical and neuropsychological evaluation. Patients' disability was measured with the EDSS questionnaire (Lechner-Scott et al., 2003). The 9 Hole Peg Test (9-HPT, both dominant-hand [DH] and non-dominant hand [NDH]) and the 25 Foot Walk Test (25-FWT) were measured according to the Multiple Sclerosis Functional Composite (MSFC) scoring manual (Cohen et al., 2001). History taking included occurrence of relapses and change in therapy, and patients completed the Multiple Sclerosis Neuropsychological Screening Questionnaire (MSNQ) (Sonder et al., 2012) and the Multiple Sclerosis Walking Scale (MSWS-12) (Hobart et al., 2003). All subjects completed the Checklist Individual Strength (CIS-20) (Vercoulen et al., 1994) and the Athens Insomnia Scale (AIS) (Soldatos et al., 2000) questionnaires within four days of the visit.

2.2. MR image acquisition

MR imaging was performed on a 3T whole-body MR scanner GE Discovery MR750 (GE Healthcare, Milwaukee, WI., USA) using an eight-channel phased-array head coil. No MRI hardware or software upgrades occurred during the study. The MR protocol included a 2D T2-weighted sequence planned perpendicular to the cervical cord (with TR/TE = 5035/85 ms, voxel size 0.4 × 0.4 × 4.0 mm, 26 consecutive slices), covering 104 mm of upper cervical cord starting at the most inferior surface of the pons.

For brain imaging, a sagittal 3D T1-weighted fast spoiled gradient echo sequence (FSPGR with TR/TE/TI = 8.2/3.2/450 ms, voxel size 1.0 × 1.0 × 1.0 mm), and a sagittal 3D T2-weighted fluid attenuated inversion recovery sequence (FLAIR with TR/TE/TI = 8000/130/2338 ms, voxel size 1.0 × 1.0 × 1.2 mm) with full coverage of the cerebrum and cerebellum, as well as (part of) the upper cervical cord.

2.3. MR image analysis

The upper cervical cord was segmented from the T2-weighted images using SCT-PropSeg (De Leener et al., 2014; Gros et al., 2019), a fully automated method incorporated in Spinal Cord Toolbox version 4.2. In a few cases the automatic detection of the cervical cord level failed and the label was selected by the operator using the sct_label_vertebrae option *initz*. A careful visual examination of segmentation output was performed and in case of failure, the segmentations were excluded from further analysis. Due to bending of the cord, C3 and C4 area could not be measured in all subjects at all time points. Therefore, we calculated UCCA for C1 through C4 separately.

One rater (SZ) manually delineated and segmented the cord lesions using ITK- SNAP Toolbox 3.6.0 (Yushkevich et al., 2006) under the supervision of an expert neuroradiologist (BM). Lesions were identified as focal hyperintensities on T2-weighted axial images, well demarcated from the surrounding normal-appearing tissue. To avoid false positives, a conservative approach was followed in which lesions were selected only if they were visible on at least two consecutive axial slices and could not be attributed to partial volume effects. The anatomical image was registered to the SC MRI template PAM50 and the T2 cervical cord lesion volume (CCLV) computed using the command line *sct_analyse_lesion*.

Details on brain imaging analysis can be found in **Supplemental Methods**.

2.4. Statistical analysis

Statistical analyses were performed using SPSS26 (IBM SPSS, Chicago, USA). Shapiro-Wilk test was used to assess the normally distribution of the variables. Group comparisons for baseline demographics were performed by independent samples *t*-test, Mann Whitney *U test*, or Chi Square test, when appropriate.

Since not all subjects had an equal number of measurements (i.e. fewer time points and/or fewer cord levels reliably analysed), linear mixed model (LMM) analysis was used to investigate: (a) differences in UCCA between HC and RRMS; (b) the relation between UCCA and the presence of lesions over the upper cervical cord; and (c) the relation between UCCA and CCLV at a given cervical level. Details on the LMM analyses are given in the **Supplemental Methods**. In short, the relation between UCCA and CCLV was investigated using two approaches: (1) over all cervical levels (i.e. RRMS subjects with a lesion at any cervical level, referred to as "MS+CL", versus RRMS subjects without any lesion at any cervical level, "MS-CL"); and (2) for each cervical level separately (e.g., in relation to UCCA at the C1 level, only those patients with lesions at the C1 level were classified as having a lesion, and similarly for C2, C3 and C4). This second approach allowed an anatomical refinement of the LMM in the previous analysis by tying the UCCA to the presence or absence of cord lesions at the same level.

In addition, LMM was also used to investigate the relation between disability and UCCA in all RRMS subjects, and between disability and cervical lesions (presence and/or volume) in the MS+CL subjects (for details, see **Supplemental Methods**).

3. Results

3.1. Demographics

In total, 40 subjects with early RRMS and 15 age-and-sex matched HCs were included in the study. Data from 6 subjects were excluded from the analysis, because they had only a baseline examination ($n = 2$ loss to follow-up, $n = 2$ switch to second-line therapy), or because SCT-PropSeg failed at baseline ($n = 2$). The baseline demographics of the final cohort of 13 HCs and 36 MS subjects are depicted in Table 1. Based on the presence of lesions in the upper cervical cord (C1 through C4) at baseline, the patient group was subdivided in MS-CL (i.e. without any

Table 1
Baseline demographics of HC, MS and MS-CL and MS+CL subjects.

	HC ($n = 13$)	**MS** ($n = 36$)	**MS-CL** ($n = 20$)	**MS+CL** ($n = 16$)
Age in years, mean ± SD	37.3 ± 12.2	35.6 ± 7.6	34.4 ± 7.7	37.0 ± 7.4
Sex, m/f (% m)	4/9 (31)	7/29 (19)	1/19 (5) *	6/10 (38)
Disease duration in years, mean ± SD	–	2.5 ± 1.3	2.3 ± 1.4	2.7 ± 1.4
ARR, median (range)	–	0 (0–2)	0 (0–2)	0 (0–2)
Treatment[1], *n* (%)	–			
none		8 (22)	3 (15)	5 (31)
interferon		4 (11)	2 (10)	2 (13)
glatiramer acetate		5 (17)	4 (20)	2 (13)
dimethyl fumarate		13 (36)	7 (35)	6 (38)
teriflunomide		2 (6)	1 (5)	1 (6)
Treatment duration in years, mean ± SD (range)	–	1.7 ± 1.1	1.7 ± 1.1	1.9 ± 1.2
EDSS, median (range)	–	3.0 (2.5–3.5)	3.0 (2.5–3.5)	3.0 (1.5–3.5)
9-HPT in seconds, mean ± SD				
dominant hand	–	19.0 ± 2.1	18.5 ± 2.0	19.6 ± 2.2
non-dominant hand		20.5 ± 2.8	20.1 ± 2.1	21.0 ± 3.4
25-FWT in seconds, mean ± SD	–	4.4 ± 1.2	4.5 ± 1.2	4.3 ± 1.4
CIS, median (IQR)	52 (38–64)	82 (51–92) **	85 (70–92)	59 (37–92)
AIS, median (IQR)	2 (2–4)	5 (3–6) **	5 (3–8)	4 (2–6)
MSWS, median (IQR)	–	18 (14–29)	18 (16–30)	17 (12–26)
MSNQ, median (IQR)	–	25 (15–33)	25 (20–31)	20 (8–35)
NBV in ml, mean ± SD	1538 ± 31	1530 ± 52	1517 ± 56	1545 ± 45
NLV in ml, mean ± SD	1.39 ± 3.32	4.68 ± 3.98 **	4.09 ± 3.72	5.42 ± 4.28

Legend: abbreviations: SD = standard deviation; ARR = annualized relapse rate; EDSS = expanded disability status scale; 9-HPT = 9-hole peg test; 25-FWT = 25-foot walk test; CIS = checklist individual strength; AIS = Athens insomnia scale; MSWS = multiple sclerosis walking scale; MSNQ = multiple sclerosis neurological screening questionnaire; NBV = normalized brain volume; NLV = normalized brain lesion volume; IQR = interquartile range; FU = follow-up. Statistics: * $p \leq 0.05$; ** $p \leq 0.01$.

[1] Treatment group interferon consists of interferon beta-1a (Avonex®, Rebif®), beta-1b (Betaferon®) and peginterferonbeta-1a (Plegridy®); other treatments are glatiramere acetate (Copaxone®), dimethyl fumarate (Tecfidera®), and teriflunomide (Aubagio®).

lesions, $n = 20$ subjects) and MS+CL (i.e. with lesions in at least one cord level, $n = 16$ subjects).

At baseline, subjects with MS had significantly higher normalized brain lesion volume (NLV, $p = 0.008$), CIS scores ($p = 0.004$) and AIS scores ($p = 0.004$) than HCs. The MS-CL group contained significantly fewer males than the MS+CL group ($p = 0.030$), but no other baseline differences were seen between the two MS groups.

One HC (female) was unable to undergo MRI at year-1 but had a scan at year-2. Five MS subjects (female, all from MS-CL group) only had baseline and year 1, but not year-2, measurements ($n = 3$ switch to second-line therapy; $n = 1$ unable to undergo MRI; $n = 1$ lost to follow-up).

3.2. Upper cervical cord area, lesions and lesion volume

Table 2 lists the UCCA in HC and MS subjects at each time point, and the change from baseline to year-2. Values are provided for the entire C1:C4 cord length (for those subjects in which this was available) and for each level separately. Within the MS group, we listed the number of patients without and with lesions at a specific cord level, and the UCCA of each group at that cord level, and CCLV when applicable.

3.2.1. Differences in UCCA between HC and MS subjects

LMM analysis showed that UCCA was lower in MS subjects than in HC ($B = 6.203$, SE=1.975, $p = 0.003$). In addition, UCCA reduced over time from year-0 to year-2 ($B = 0.986$, SE=0.390, $p = 0.012$) and from year-1 to year-2 ($B = 0.690$, SE=0.348, $p = 0.048$) in the entire cohort, but there was no interaction between time and subject type (i.e. HC or MS, $p \geq 0.144$). Neither sex nor age was a significant variable in the model.

3.2.2. Relation between UCCA (change) and the presence of lesions in the upper cervical cord in MS subjects

No difference in baseline UCCA was seen between patients who had at least one lesion in the entire C1-C4 region (MS+CL) and those who did not (MS-CL) ($B=-0.009$, SE=2.264, $p = 0.997$). Considering this comparison at each cord level separately, similarly, UCCA did not differ between patients with lesions at that level and those without ($B=-0.271$, SE=0.657, $p = 0.681$). This was true for all time points, and neither sex nor age influenced these results.

Looking at change over time, UCCA change (Δ from year-0 to year-2) was independent of the presence of cord lesions at baseline for all cervical levels ($p = 0.996$ for MS-CL vs MS+CL, and $p = 0.391$ for subjects with and without lesions at a given cervical level, respectively). Neither sex nor age influenced these results.

3.2.3. Relation between UCCA (change) and CCLV (change) by cervical level in MS subjects

In MS subjects with cord lesions at the respective cord level, UCCA was independent of CCLV ($B=-0.008$, SE=0.007, $p = 0.278$) and CCLV was independent of UCCA ($B=-0.428$, SE=1.990, $p = 0.830$) over all cervical levels. No effects of age, sex or time were found for either analysis.

In addition, UCCA change over time (Δ year-0 to 2) was independent of baseline CCLV across all levels (Fig. 1a, $B=-0.001$, SE=0.004, $p = 0.889$), or in any of the cervical levels separately (data not shown). Furthermore, UCCA change over the second year (Δ year-1 to 2) was independent of CCLV change over the first year (Δ year-0 to 1) (Fig. 1b, $B=-0.001$, SE=0.004, $p = 0.872$). Sex, age and cervical level did not influence these results.

3.3. Relations between cervical cord area and cervical cord lesions with disability over time

Table 3 provides the disability measures in the overall MS-CL and MS+CL groups for each time point. One subject (male, MS+CL group) was unable to complete the 9-HPT correctly at multiple time points, and was therefore excluded from the 9-HPT analyses.

LMM analysis over all cervical levels and all time points showed no significant differences between the MS-CL and MS+CL groups for any of

Table 2
Upper cervical cord area (UCCA) in the four cervical levels for HC and MS, as well as split for MS subjects without or with lesions in a given cervical level, as well as lesion volume in the latter.

	HC		MS		MS without lesions in given cervical level		MS with lesions in given cervical level		
Cervical level	*n*	UCCA (mm^2) mean ± SD	*n*	UCCA (mm^2) mean ± SD	*n*	UCCA (mm^2) mean ± SD	*n*	UCCA (mm^2) mean ± SD	CCLV (μl) median (IQR)
C1									
year 0	13	76.76 ± 6.99	36	71.71 ± 6.29	24	71.47 ± 5.29	12	72.20 ± 8.19	35.08 (14.04–66.40)
year 1	12	78.17 ± 5.97	35	71.39 ± 6.62	25	71.43 ± 6.28	10	71.31 ± 7.77	33.68 (17.20–159.90)
year 2	13	76.11 ± 7.21	31	70.84 ± 6.38	21	70.81 ± 5.68	10	70.90 ± 8.01	40.53 (12.38–111.19)
Δ y0–2	13	−0.65 ± 2.26	31	−1.07 ± 1.72	21	−0.70 ± 1.68	10	−1.87 ± 1.61	+5.24 (−49.88–82.19)
C2									
year 0	13	76.75 ± 7.30	36	70.87 ± 6.22	24	70.70 ± 5.72	12	71.20 ± 7.39	106.73 (58.45–154.41)
year 1	12	77.77 ± 6.86	36	71.00 ± 6.05	25	71.38 ± 5.79	11	70.13 ± 6.82	79.27 (12.62–141.67)
year 2	13	76.65 ± 6.82	31	69.97 ± 6.74	15	71.49 ± 6.30	16	68.55 ± 7.02	46.30 (29.97–87.02)
Δ y0–2	13	−0.10 ± 2.08	31	−1.02 ± 1.67	15	−0.83 ± 1.70	16	−1.20 ± 1.68	+15.15 (−63.34–34.42)
C3									
year 0	12	78.11 ± 7.60	35	72.56 ± 6.25	23	73.88 ± 6.04	12	70.03 ± 6.08	23.99 (14.34–213.37)
year 1	10	79.59 ± 8.12	31	72.43 ± 6.59	19	73.22 ± 6.23	12	71.17 ± 7.22	76.88 (32.26–155.87)
year 2	11	78.40 ± 7.41	27	71.84 ± 7.05	15	73.02 ± 7.00	12	70.37 ± 7.13	68.25 (31.77–228.43)
Δ y0–2	11	+0.35 ± 2.22	26	−0.83 ± 2.10	14	−0.41 ± 2.38	12	−1.32 ± 1.69	+10.29 (−8.75–56.95)
C4									
year 0	8	80.15 ± 6.94	26	74.75 ± 5.35	20	74.66 ± 5.80	6	75.03 ± 3.92	47.87 (10.58–167.44)
year 1	8	81.47 ± 6.58	29	74.63 ± 5.41	25	74.89 ± 5.79	4	72.99 ± 1.09	37.85 (10.35–158.98)
year 2	6	78.91 ± 5.69	20	73.58 ± 7.08	14	73.27 ± 6.81	6	74.28 ± 8.30	37.05 (15.35–73.94)
Δ y0–2	6	+0.42 ± 2.01	16	−0.62 ± 3.90	12	−0.99 ± 3.16	4	+0.50 ± 6.09	−11.94 (−57.65–25.22)

Legend: abbreviations: SD = standard deviation, IQR = inter-quartile range. Please note that values for Δ y0–2 are calculated from subjects with data at the given cervical level available at both year 0 and year 2, indicated by *n*, which may deviate from the arithmetic difference of the means at year 0 and year 2 for all available subjects.

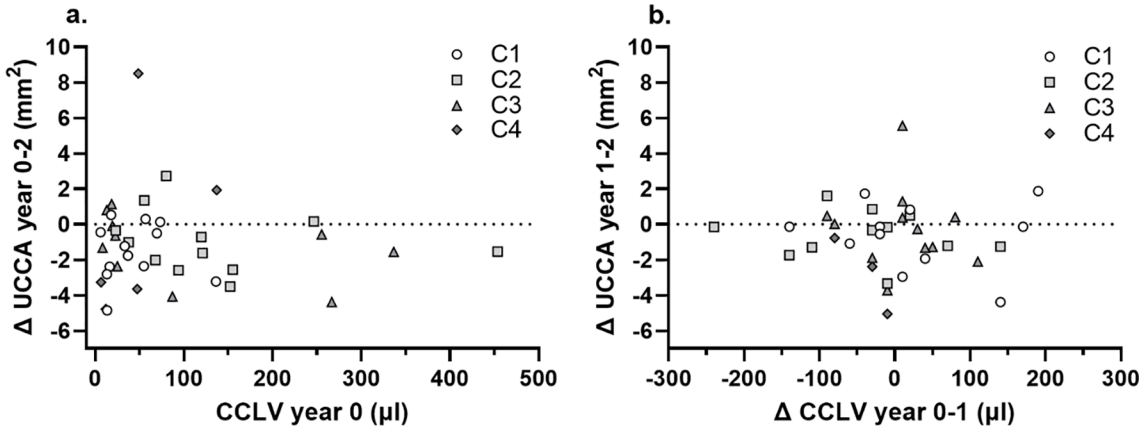

Fig. 1. Relationship between CCLV (μl) and UCCA (mm^2) over four cervical levels. (a) Relation between CCLV year 0 and UCCA from year-0 to year-2; (b) relation between CCLV change over the first year and UCCA change over the second year.

the disability measures, except for a trend towards slightly higher EDSS scores in the MS-CL group compared to the MS+CL group ($B = 0.831$, SE=0.424, $p = 0.058$).

3.3.1. Relation between disability and UCCA in MS subjects

Lower UCCA was related to higher 9-HPT-NDH times (i.e., poorer performance; $B=-0.072$, SE=0.020, $p<0.001$) (Fig. 2a), with a significant contribution from age ($B = 0.131$, SE=0.048, $p = 0.010$). UCCA was not associated with any of the other disability measures (EDSS, 9-HPT DH, 25-FWT, CIS, AIS or MSWS scores). However, a trend was seen for higher MSNQ outcome in MS subjects with lower UCCA ($B=-0.134$, SE=0.080, $p = 0.095$).

3.3.2. Relation between disability and cervical lesions (presence and/or volume) in MS subjects

Using the cord level-specific classification into patients with or without lesions, EDSS did not differ between MS subjects with lesions at that level and those without ($p = 0.879$). A group * time interaction was found, where subjects without lesions at a specific level performed better than subjects with lesions for 9-HPT NDH scores over time (year-1 to year-2) ($B = 0.549$, SE=0.253, $p = 0.031$) as shown in Fig. 2b, but age significantly affected this relationship ($B = 0.127$, SE=0.048, $p = 0.012$).

A similar interaction was found for 25-FWT outcome (Fig. 2c), where subjects without lesions in a specific cervical level also performed better over time than subjects with lesions ($B = 0.330$, SE=0.150, $p = 0.029$ for year-0 to year-2 and $B = 0.418$, SE=0.153, $p = 0.006$ for year-1 to year-2). Across all time points, the groups did not differ significantly from each other regarding the 25-FWT, although a trend was observed ($B=-0.238$, SE=0024, $p = 0.080$). Other disability measures CIS, AIS, MSWS and MSNQ did not differ between patients with or without lesions in a specific cervical level.

Looking at disability progression over time in patients with lesions at a specific cervical cord level, we found that 9-HPT progression over time was not influenced by baseline CCLV (Fig. 3a,b), but it was associated with baseline UCCA (Fig. 3c,d) (DH: $B=-0.056$, SE=0.028 $p = 0.051$; NDH: hand $B=-0.066$, SE=0.027, $p = 0.015$), where lower baseline UCCA was predictive of poorer 9-HPT performance. EDSS, CIS, AIS,

Table 3
Disability measures in MS-CL and MS+CL subjects at year 0, year 1, year 2 and over time (Δ y0–2).

	MS-CL		MS+CL			MS-CL		MS+CL	
EDSS	***n***	**median (IQR)**	***n***	**median (IQR)**	**CIS**	***n***	**median (IQR)**	***n***	**median (IQR)**
year 0	20	3.0 (2.5 – 3.5)	16	3.0 (1.5 – 3.5)	year 0	20	85 (70 – 92)	16	59 (37 – 92)
year 1	19	3.0 (2.5 – 3.5)	16	3.5 (1.5 – 3.5)	year 1	19	82 (61 – 96)	16	64 (41 – 85)
year 2	15	3.5 (2.5 – 3.5)	16	3.0 (1.5 – 3.5)	year 2	15	75 (61 – 92)	16	63 (46 – 86)
Δ y0-2	15	+0.5 (-0.5 – 1.0)	16	0.0 (-0.5 – 0.0)	Δ y0-2	15	-5 (-18 – 8)	16	-1 (-18 – 24)
9-HPT, DH	***n***	**mean ± SD**	***n***	**mean ± SD**	**AIS**	***n***	**median (IQR)**	***n***	**median (IQR)**
year 0	20	18.5 ± 2.0	16	19.6 ± 2.2	year 0	20	5 (3 – 8)	16	4 (2 – 6)
year 1	20	18.6 ± 1.5	16	19.6 ± 4.2	year 1	19	5 (3 – 9)	16	4 (2 – 5)
year 2	15	18.3 ± 1.8	16	19.4 ± 4.0	year 2	15	6 (4 – 9)	16	3 (2 – 8)
Δ y0-2	15	-0.23 ± 1.74	16	-0.14 ± 2.68	Δ y0-2	15	0 (0 – 2)	16	1 (-2 – 2)
9-HPT, NDH	***n***	**mean ± SD**	***n***	**mean ± SD**	**MSWS**	***n***	**median (IQR)**	***n***	**median (IQR)**
year 0	20	20.1 ± 2.1	16	21.0 ± 3.4	year 0	20	18 (16 – 30)	16	17 (12 – 26)
year 1	19	20.0 ± 2.0	16	20.4 ± 3.7	year 1	19	21 (15 – 27)	16	16 (13 – 26)
year 2	15	19.3 ± 2.5	16	20.6 ± 4.3	year 2	15	21 (14 – 27)	16	14 (12 – 23)
Δ y0-2	15	-0.69 ± 1.75	16	-0.44 ± 2.10	Δ y0-2	15	0 (-7 – 5)	16	0 (-3 – 1)
25-FWT	***n***	**mean ± SD**	***n***	**mean ± SD**	**MSNQ**	***n***	**median (IQR)**	***n***	**median (IQR)**
year 0	20	4.5 ± 1.2	16	4.3 ± 1.4	year 0	20	25 (20 – 31))	16	20 (8 – 35)
year 1	19	4.5 ± 1.1	16	4.2 ± 1.3	year 1	19	26 (19 – 32)	16	19 (11 – 35)
year 2	15	4.9 ± 2.1	16	4.9 ± 2.4	year 2	15	23 (16 – 30)	16	19 (9 – 34)
Δ y0-2	15	+0.30 ± 0.90	16	+0.58 ± 1.23	Δ y0-2	15	-2 (-8 – 2)	16	0 (-9 – 6)

Legend: 9-HPT and 25-FWT in seconds; abbreviations SD = standard deviation; IQR = inter-quartile range.

MSWS and MSNQ progression over time could not be explained by baseline UCCA, and none of the disability outcomes could be explained by baseline CCLV.

4. Discussion

In this study, we investigated the cross-sectional and longitudinal relationship between upper cervical cord area and lesions in subjects with early RRMS. The study design and statistical analyses presented here allowed us to investigate the longitudinal dynamics and relations between cervical cord lesions (presence or volume) and cervical cord area, both globally (i.e. over the C1:C4 area) and locally (i.e. per cervical level).

We found a lower UCCA in subjects with RRMS compared to HCs but decrease of UCCA over time occurred independent of group. Moreover, UCCA was not related to the presence or volume of cervical cord lesions (CCLV), neither cross-sectionally nor longitudinally. While we found a relation between lower baseline UCCA and faster worsening of 9-HPT-NDH and 25-FWT scores over time, there was no relation between disability change over time and the presence or volume of lesions in the upper cervical cord.

We found no relation between the presence or volume of cervical cord lesions and UCCA at any time point (cross-sectional) or considering their change over time (longitudinal). Although some studies have reported these associations in progressive MS types (Pravata et al., 2020; Valsasina et al., 2018; Petrova et al., 2018), in RRMS and CIS this association has not been found (Valsasina et al., 2021; Zurawski et al., 2019). These results seem to be confirmed by post-mortem studies, which observed that neither lesion size nor lesion number in the SC correlated with the degree of local atrophy of the cord when correcting

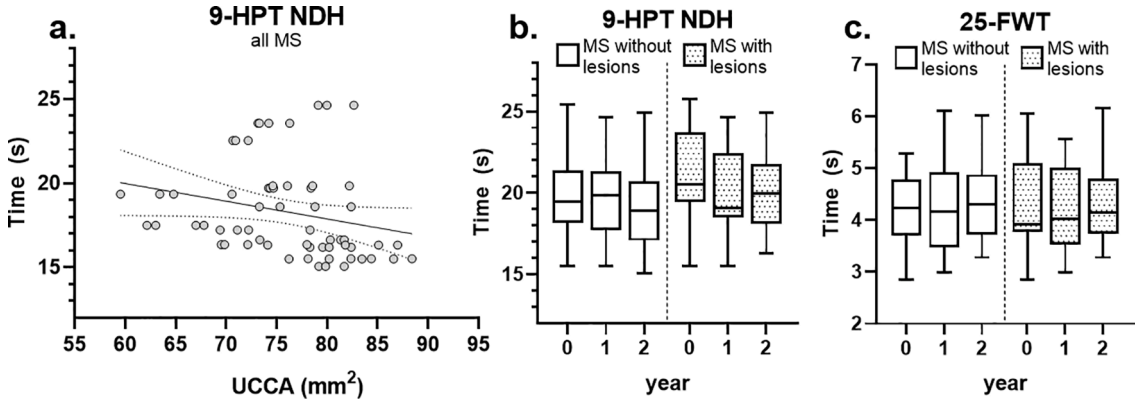

Fig. 2. Disability in MS subjects. (a) 9-HPT NDH outcome with regard to UCCA shows a negative relation between the two; (b) Subjects without lesions in a given cervical level (white) have lower 9-HPT non-dominant hand times from year-1 to year-2 when compared to subjects with lesions in a given cervical level (dotted); (c) Comparable results are seen for 25-FWT, where subjects with lesions in a given cervical level (dotted) have a greater increase in 25-FWT time from year-0 to year-2 than subjects without lesions in a given cervical level (white).

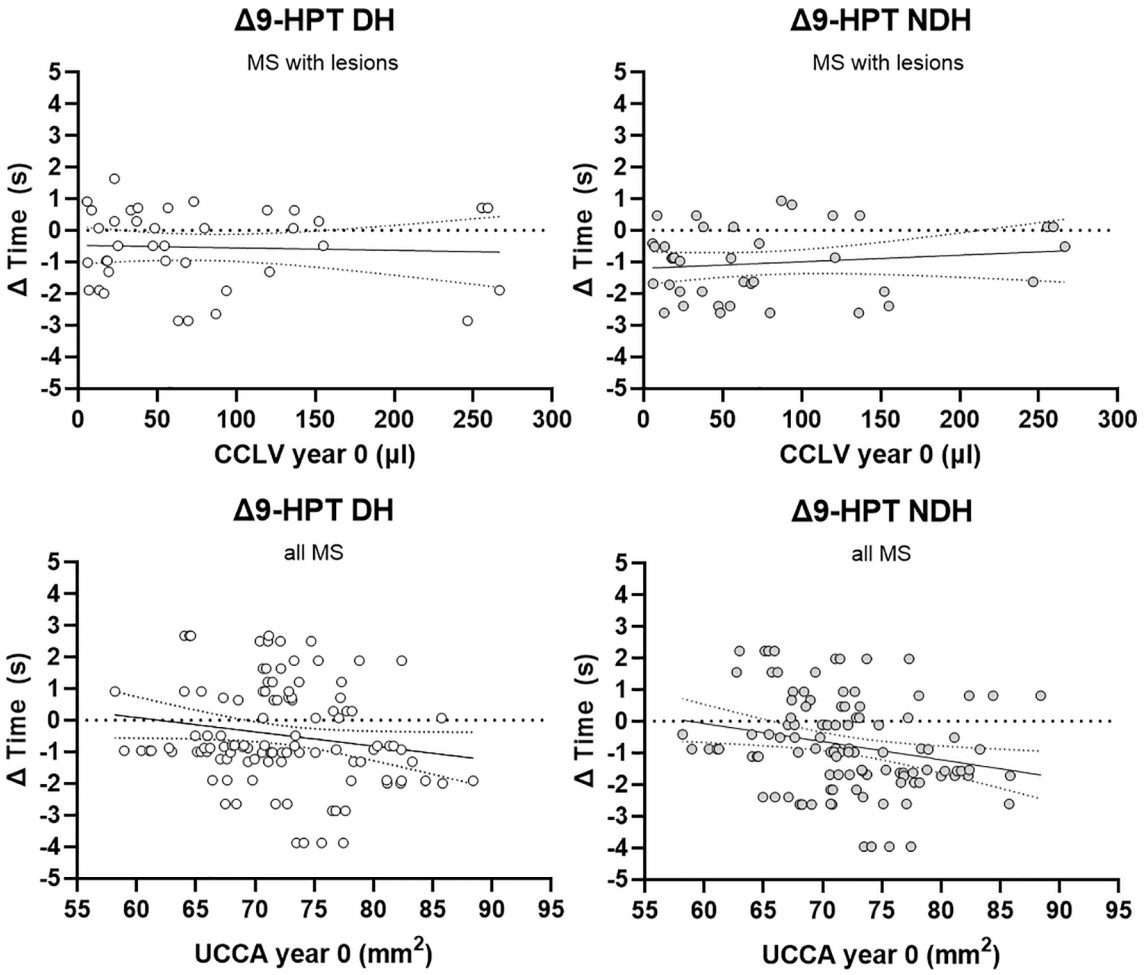

Fig. 3. 9-HPT progression over time from the dominant (white circles) and non-dominant (grey circles) hand shows no relation with baseline CCLV (top), but a negative relation with baseline UCCA (bottom).

for disease duration (Evangelou et al., 2005; Gilmore et al., 2009). Results of several *in vivo* cord studies confirmed this, by showing that local demyelination (Lee et al., 2021), increased diffusivity (Wolanczyk et al., 2020) and decreased axonal and neurite density (Pravata et al., 2020; Collorone et al., 2020), rather than lesion size or number, were substrates of decreased UCCA in MS.

RRMS subjects with lower baseline UCCA showed subsequent performance worsening on 9-HPT-NDH (upper extremity function) and 25-FWT (lower extremity function) over time in our study, which was also found previously (Lukas et al., 2015; Zurawski et al., 2019). UCCA was not related to EDSS scores in our study, in line with previous studies in non-progressive MS (Kearney et al., 2015; Brownlee et al., 2017; Pravata et al., 2020; Valsasina et al., 2021; Kerbrat et al., 2020; Lin et al., 2003). These results underline the importance of investigating the different functional systems when looking at the relation between disability and UCCA in early MS. It appears that in early MS, cervical cord atrophy is correlated to pyramidal, and mainly manual, impairment (Valsasina et al., 2018; Valsasina et al., 2021; Zurawski et al., 2019), rather than overall disability (i.e., EDSS). We found no associations between CCLV and disability in our early MS cohort, where this relation was present in more progressive MS phenotypes with moderate to severe disability (Kearney et al., 2015; Kerbrat et al., 2020; Eden et al., 2019) or long-standing MS (Dekker et al., 2020). Interestingly, the relationship of lower UCCA with worsening upper extremity function has also been reported in spinal cord injury due to other causes (Freund et al., 2011), which seems to support the hypothesis that overall spinal cord damage rather than MS-related inflammation (e.g., CCLV) may be the physiological basis of upper extremity disability.

Our result that subjects with RRMS exhibited lower UCCA compared to HC is in line with most studies (Biberacher et al., 2015; Evangelou et al., 2005; Valsasina et al., 2018, 2021), although some studies did not find a difference in UCCA between RRMS and HC subjects (Klein et al., 2011; Rocca et al., 2011). An important factor in these differences is disease duration (Evangelou et al., 2005; Valsasina et al., 2021), as well as possible swelling in the cervical cord due to inflammation (Valsasina et al., 2021; Brex et al., 2001), which may increase UCCA, especially in earlier MS stages such as clinically isolated syndrome (CIS) (Biberacher et al., 2015); since we studied subjects early in their disease course, this may have played a role in our results as well. In addition, the cervical cord atrophy rate appears to be similar for HC and RRMS subjects (Brex et al., 2001) and therefore seems to accelerate mostly in the progressive stages of the disease (Klein et al., 2011; Rocca et al., 2011; Zeydan et al., 2018).

This study has a few limitations. The MS+CL group had significantly fewer males, therefore we included sex in all statistical analyses. Furthermore, bending of the cervical cord makes it difficult to acquire C3 and C4 results in all subjects, therefore LMM analysis was used to overcome the problem of missing data. In addition, did not use the generalized boundary-shift integral (GBSI) for the longitudinal cord atrophy measurements, but instead used LMM analysis to minimize subject-specific measurement variability. Last, it is important to note that our relatively small cohort consisted of subjects with a low degree of disease activity, treated only with first-line immunomodulatory therapies or no treatment at all. These inclusion criteria allow to study *in vivo* biological phenomena with little or no effect of therapy, but potentially

exclude those patients in whom the pathological mechanisms occurring in MS (including neurodegeneration and neuroinflammation) are more pronounced.

5. Conclusion

In conclusion, we found that there is no relation between cervical cord lesions (presence and/or volume) and cervical cord atrophy in subjects with early RRMS, neither cross-sectionally nor longitudinally. In addition, RRMS subjects with lower UCCA values appear to worsen in both upper and lower extremity function over time. Results should be replicated over longer follow-up periods.

Term	Definition
Conceptualization	Ideas; formulation or evolution of overarching research goals and aims
Methodology	Development or design of methodology; creation of models
Software	Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components
Validation	Verification, whether as a part of the activity or separate, of the overall replication/ reproducibility of results/ experiments and other research outputs
Formal analysis	Application of statistical, mathematical, computational, or other formal techniques to analyse or synthesize study data
Investigation	Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection
Resources	Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools
Data Curation	Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later reuse
Writing - Original Draft	Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation)
Writing - Review & Editing	Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision–including pre-or postpublication stages
Visualization	Preparation, creation and/or presentation of the published work, specifically visualization/ data presentation
Supervision	Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team
Project administration	Management and coordination responsibility for the research activity planning and execution
Funding acquisition	Acquisition of the financial support for the project leading to this publication

CRediT authorship contribution statement

Merlin M. Weeda: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Sofia Zywicki:** Methodology, Validation, Formal analysis, Investigation, Writing – review & editing. **Iman Brouwer:** Software, Validation, Writing – review & editing. **Bastiaan Moraal:** Methodology, Validation, Investigation, Writing – review & editing. **Joep Killestein:** Resources, Writing – review & editing, Supervision. **Paolo Gallo:** Writing – review & editing, Supervision. **Frederik Barkhof:** Resources, Supervision, Writing – review & editing, Project administration, Funding acquisition. **Petra J.W. Pouwels:** Conceptualization, Validation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Hugo Vrenken:** Conceptualization, Validation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2022.103713.

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