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ORIGINAL COMMUNICATION

Relevance of early cervical cord volume loss in the disease evolution of clinically isolated syndrome and early multiple sclerosis: a 2-year follow-up study

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Abstract Upper cervical cord area (UCCA) atrophy is a prognostic marker for clinical progression in longstanding multiple sclerosis (MS). The objectives of the study were to quantify UCCA atrophy and evaluate its impact in clinically isolated syndrome (CIS) and relapsing–remitting MS (RRMS); to compare converting CIS patients with stable CIS, and to study changes of UCCA and brain white matter (WM) and grey matter (GM) at 2-year follow-up. 110 therapy-naive patients including 53 CIS [6 ± 6 months after symptom onset (SO)] and 57 early RRMS (SO: 12 ± 9 months) underwent sagittal 3D-T1w brain MR (3T). Mean UCCA (C1–C3 level), WM and GM, disability status (EDSS), pyramidal and sensory functional scores, motoric fatigue were assessed at baseline (BL), 12 and 24 months. Volumes were compared with 34 age- and gender-matched healthy controls to assess atrophy. RRMS (78.1 ± 8.7 mm^2, $p = 0.011$) and converting CIS (77.3 ± 8.0 mm^2, $p = 0.046$) presented with baseline UCCA atrophy, when compared with controls (82.7 ± 5.2 mm^2), but not stable CIS (82.6 ± 7.4 mm^2, $p = 0.998$). Baseline WM was reduced in RRMS (509.3 ± 25.7 ml vs. controls: 528.4 ± 24.1 ml, $p = 0.032$). Baseline UCCA correlated negative with muscular weakness and fatigability in all patients and RRMS. EDSS exceeding 3 was associated with lower baseline UCCA. Longitudinal atrophy rates were higher in UCCA than in brain volumes. Early cervical cord atrophy in CIS and RRMS was confirmed and may represent a potential new risk marker for conversion from CIS to MS. Baseline atrophy and atrophy change rates were higher in UCCA compared to WM and GM, suggesting that cervical cord volumetry might become an additional MRI marker relevant in future clinical studies in CIS and early MS.

Keywords Multiple sclerosis · Clinically isolated syndrome · RRMS · Cervical cord · Brain · Conversion

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Introduction

Spinal cord (SC) pathology plays an important role in diagnosis and development of clinical impairment in multiple sclerosis (MS) [1]. The presence of SC lesions is an established criterion in recent diagnostic guidelines [2–8]. Reduction in SC volume or mean cross-sectional area has been previously suggested as a potential outcome measure to monitor disease progression in clinically definite MS [4–8]. However, longitudinal data on the temporal dynamics of SC atrophy in patients with clinically isolated syndrome (CIS) and early MS are sparse. Previous studies mainly investigated differences between relapsing–remitting (RRMS) and progressive MS subtypes [6, 9–14] with

inconsistent results regarding the onset of SC atrophy during disease evolution [8, 9, 15–17]. This also holds true for CIS, as only two studies examined the longitudinal course of cervical cord atrophy in such patients. In a 1-year follow-up study SC atrophy in CIS was noted only in patients with brain lesions [15], a recent longitudinal study over 5-years suggested spinal cord atrophy as an important marker for disability progression in non-spinal CIS patients [18].

The present study aimed at investigating the clinical impact of SC atrophy in early phases of the disease as well as its prognostic relevance in CIS patients in terms of predicting the risk of conversion to clinically definite MS (CDMS) during 2 years of follow-up. Since several recent studies have found early brain involvement in CIS patients [13, 19, 20], we additionally aimed at comparing longitudinal SC results to global brain grey and white matter (GM, WM) atrophy, both cross-sectionally and longitudinally.

Materials and methods

Patients and healthy controls

53 CIS and 57 RRMS patients were enrolled from an ongoing longitudinal study of the German Competence Network Multiple Sclerosis (KKNMS, http://www.kompetenznetz-multiplesklerose.de). Inclusion criteria of the study were: adult patients, therapy-naive to disease-modifying drugs, diagnosis of CIS with a high risk of conversion to MS, or early CDMS less than 3 years after the first documented symptoms have occurred. Patients with a CIS within 6 months prior to baseline visit were eligible when either 3 of 4 Barkhof criteria were fulfilled or 2 of 4 Barkhof criteria were fulfilled and altered visually evoked potentials or the detection of oligoclonal bands in the cerebrospinal fluid supported the diagnosis. The diagnosis for CDMS was based on the revised McDonald criteria 2005 [3].

High-resolution magnetic resonance imaging (MRI) and assessment of clinical disability was performed at yearly visits including the Expanded Disability Status Scale (EDSS) [21], the pyramidal sensory and bowel–bladder Functional System Scores (FSS) and the motoric fatigue score which is part of the fatigue scale for motor and cognitive functions (FSMC) [22]. Patient-reported restrictions of the walking abilities were classified into five groups (unrestricted, >500, >300, >200, >100 m).

Standard disease-modifying treatment (DMT by interferon beta, glatiramer acetate or dimethyl fumarate) was initiated after inclusion in the study in 72% of CIS and 84% of RRMS patients.

The baseline (BL) visit and MRI took place 9 ± 8 months (mean $\pm$ standard deviation, SD) after the onset of symptoms. The first follow-up (FU1) visit and MRI were acquired after 11 ± 3 and 26 ± 6 months for the second follow-up (FU2). Of the 110 patients studied at baseline, 92 patients completed FU1 (43 CIS patients and 49 RRMS patients) and 63 patients completed FU2 (29 CIS patients and 34 RRMS patients). For comparison of volumetric results and assessment of atrophy MRI datasets of an existing in-house database of 34 gender- and age-matched healthy volunteers (HC) were evaluated. The HC group was examined at the same scanner using the identical imaging protocol as the patient group.

MR imaging

Imaging was performed on a 3T scanner using a standardized brain imaging protocol including sagittal, isotropic ($1 \times 1 \times 1$ mm^3) 3D-T1-weighted and 3D-FLAIR sequences covering the upper cervical cord. Dedicated cord images were not acquired. Details of the MRI protocol are provided in the electronic supplement (Online Resource 1).

Quantitative image analysis

Prior to analysis, images of all included subjects were anonymized and randomly presented to a single rater (I.H.) performing measurements of mean upper cervical cord area (UCCA) between the C1 and C3 level based on sagittal 3D-T1-weighted image series. UCCA was measured using a semi-automated software (NeuroQLab; Fraunhofer MEVIS, Bremen, Germany) as previously described [7]. The method has been previously referred to as both MUCCA and UCCA in different studies; it does, however, refer to the same technique [5, 7]. Details are provided in the electronic supplement (Online Resource 2).

After lesion filling, using the lesion segmentation toolbox (LST toolbox) [23], volumes of brain GM and WM were analyzed using the VBM8 pre-processing and segmentation tools with default parameters (available at http://www.fil.ion.ucl.ac.uk/spm/; http://dbm.neuro.uni-jena.de/vbm/) and normalized to the corresponding intracranial cavity volumes (ICCV) [24]. The longitudinal results were normalized to the baseline ICCV to avoid potential influence by longitudinal variations of the intracranial cavity volume.

Since no significant dependencies of UCCA on the intracranial cavity volume were observed in the control group (data not shown), uncorrected UCCA results were used for the further analyses.

In addition, brain and cervical cord results were checked for dependency on physiological aging. Age correction was necessary for brain GM (Online Resource 3); uncorrected values were used for all other variables.

Lesion quantification

Hypointense T1 lesions in the upper cervical cord were counted and scored [(1) no lesions, (2) lesion involvement by small focal lesions, (3) large and/or progressive lesions (during follow-up)]. Total brain lesion load (ml) was quantified on 3D FLAIR images using the LST tool as described [23]. The presence of significant brain lesions was scored using a minimum threshold of 2 ml for the total brain lesion load.

Statistical analysis

Statistical analyses were performed using SPSS20 (IBM SPSS, Chicago, USA). Normal distribution of MRI results, except the brain lesion load, was confirmed by Kolmogorov–Smirnov tests. To provide normal distribution of the lesion load, a log transformation (log10) was applied. Parametric statistical tests were used for the normally distributed, linear scaled variables like the volumetry results, age, and disease duration, while non-parametrical tests were applied when ordinal variables like the disability scores were included. Group comparisons regarding all volumetry results, age, and disease duration were performed by univariate ANOVA with post hoc Games–Howell tests for pairwise comparisons. Student's t tests were calculated to investigate differences of the MRI results between two disability groups. Inter-group differences between disability scores were assessed using Kruskal–Wallis tests with post hoc pairwise Mann–Whitney U tests. Group differences in ordinal variables were characterized using cross-tables with Kendall-τ analysis. Longitudinal changes of UCCA and brain GM and WM were studied by paired t tests.

Change rates per year (%) between BL, FU1, and FU2 were calculated for each patient by dividing the difference of UCCA, GM or WM by the time difference and UCCA, GM or WM at the initial time point.

Spearman correlation analyses were used to assess associations between disability scores, demographical, and MRI data. Partial Pearson correlations, corrected for age were assessed for associations between UCCA and brain WM.

Calculations of effect sizes and statistical power were assessed using G*Power [25].

Results

Demography and clinical status (Table 1)

At baseline, CIS patients did not differ significantly from RRMS patients in terms of age [CIS/RRMS: (mean ± SD): 35.0 ± 10.5/35.7 ± 11.6 years], gender (female: 62/67%), EDSS or fraction of patients with initiation of DMT (Table 1). Time since onset of first symptoms suggestive of MS was 6 ± 6 months in CIS patients, and 12 ± 9 months in RRMS.

Patients were stratified by classification at study entry and by follow-up course: non-converting CIS, CIS converting to RRMS, or RRMS at baseline. 51% of followed patients with CIS converted to RRMS during 24 months follow-up. The percentages of patients, who received DMT after inclusion in the study was high (77%) and did not differ significantly between non-converting CIS, converting CIS and RRMS patients. Thus, stratification according to the use of medication was not investigated in the further analyses.

A minority of patients showed T1w-hypointense cervical cord lesions at baseline with a significantly higher number and proportion of large or multiple lesions in the RRMS group compared to the CIS group. When comparing the CIS subgroups we observed more hypointense T1 lesions in non-converting CIS than in converts, indication that these observed cervical cord lesions had no major impact with respect to conversion from CIS to CDMS.

In contrast, the mean total brain lesion load was low in all patient subgroups and did not differ significantly between the groups.

In the whole group, median baseline EDSS at study entry was low (median [range] 1.5 [0–4.0]), without significant group differences (Table 1). During follow-up, an EDSS increase was observed, which was not significant (EDSS at FU1: 1.5 [0–6.5], EDSS at FU2: 2.0 [0–6.5]; paired Kendall's W tests between baseline, FU1 and FU2; $p = 0.068$).

There were no signs of major spinal affection, like restricted walking abilities or bladder and bowel symptoms in any subgroup (Table 1). Furthermore, the group median values of all other baseline clinical parameters (pyramidal, sensory and bowel/bladder Functional System Scores, restrictions in walking abilities and motoric fatigue (by FSMC) were not significantly different between the subgroups.

No significant within-group longitudinal changes of these clinical parameters during the follow-up period were observed.

Cervical cord and brain volumetry at baseline

Table 2 summarizes results of cervical cord and brain MRI volumetry.

At baseline, significant reduction of UCCA was detected in RRMS and in CIS compared to HC (Table 2; Fig. 1). Stratifying CIS, converting CIS patients showed significantly lower baseline UCCA compared to HC ($p = 0.046$), while non-converters did not. UCCA group differences between patient

Table 1 Demographic and clinical characteristics of the study population at baseline

	All CIS ($n = 53$)	CIS non-converting ($n = 20$)	CIS converting ($n = 21$)	RRMS ($n = 57$)	p
Female/male	33/20	9/11	15/6	38/19	
EDSS, median [range]	1.5 [0–4]	1.5 [0–4]	1.5 [0–3.5]	2 [0–4.5]	0.566[a]
					0.512[b]
Pyramidal FSS, median [range]	1 [0–3]	1 [0–3]	1 [0–2]	1 [0–3]	0.121[a]
					0.191[b]
Sensory FSS, median [range]	1 [0–3]	1 [0–3]	0 [0–3]	1 [0–3]	0.799[a]
					0.790[b]
Bladder/bowel FSS, median [range]	0 [0–2]	0 [0–1]	0 [0–2]	0 [0–1]	0.935[a]
					0.805[b]
Walking restrictions[e], no. of patients	3	2	1	1	0.593[c]
					0.543[d]
Motoric fatigue[f], median [range]	17 [10–38]	16 [10–35]	18 [12–36]	22 [10–49]	0.151[a]
					0.315[b]
Brain lesion load/ml[g], median [range]	1.0 [0–20.4]	1.3 [0–16.2]	1.0 [0–20.4]	1.2 [0–21.7]	0.407[a]
					0.707[b]
Cervical cord lesions[h], % of patients	15%	25%	5%	33%	0.027[c] *
					0.041[d] *
Cervical cord lesion grade[i], n	45/5/3	15/3/2	20/1/0	38/8/11	0.029[c] *
					0.205[d]
DMT at baseline, % of patients	72%	75%	86%	84%	0.133[c]
					0.391[d]

EDSS Expanded Disability Status Scale, *FSS* Functional System Score, *DMT* disease-modifying therapy. Subgroups: *CIS all* all baseline CIS patients, *CIS non-converting* baseline CIS patients, who did not convert to MS during follow-up period, *CIS converting* baseline CIS patients who converted to MS during follow-up period, *RRMS* all baseline RRMS patients

p significance of group differences (*significant with $p < 0.050$)

[a] CIS–RRMS (MWU test)

[b] CIS non-converting–CIS converting–RRMS (Kruskal–Wallis tests)

[c] CIS–RRMS (Kendall-Tau c)

[d] CIS non-converting–CIS converting–RRMS (Kendall-Tau c)

[e] Low-grade walking restrictions (patient-reported): more than 500 m, but not unrestricted

[f] Motoric fatigue (FSMC), low grade: score ≥22, medium grade: score ≥27, severe: score ≥32

[g] Total brain lesion volume on 3D-FLAIR images

[h] Hypointense lesions of the upper cervical cord scored on sagittal 3D-T1 weighted brain images

[i] Cervical cord hypointense T1 lesion grade: (1) no lesions/(2) single focal lesion/(3) multiple or large or progressing lesions)

subgroups did not reach significance ($p = 0.135$). Brain WM volume was significantly lower in RRMS compared to controls ($p = 0.017$). WM differences between the patient subgroups were not significant. Baseline brain GM volume loss in comparison to healthy controls was not detected in the entire patient group and within subgroups.

Cervical cord and brain volumetry at follow-up (Table 2)

Significant longitudinal changes in UCCA were found between baseline and 12 months follow-up in RRMS and in converting CIS patients. Additionally, significant UCCA changes were found in RRMS, converting CIS and the entire CIS group when comparing baseline and 24 months, and differences between 12 and 24 months. Annualized change rates of UCCA were higher in the first year of follow-up than in the second year (all patients: −1.6% during first year; −0.7% during second year; for details see Table 2).

Longitudinal changes in brain WM were not significant during the first year, but during the second year of follow-up (in the subgroups RRMS, converting CIS and all CIS patients). Annualized change rates of WM between month 12 and 24 were −1.0% for all patients.

Table 2 Comparison of mean cervical cord area (UCCA) and brain grey (GM) and white matter (WM) in MS subgroups and controls. Volumetry results presented as mean (SD), at baseline (BL) and follow-up examinations (FU1: 12 months, FU2: 24 months)

	Healthy controls	CIS all	CIS non-converting	CIS converting	RRMS
BL UCCA/mm^2	82.7 (5.2)	79.4 (7.4)	82.6 (7.1)	77.3 (8.0)	78.1 (8.7)
Inter-group	**0.019**[b]; **0.006**[c]	**0.046**[d]	0.998[g]	**0.046**[h]	**0.006**[e]
		0.784[f]	0.119[j]	0.135[k]	**0.011**[i]
					0.984[l]
n	34	53	20	21	57
FU1 UCCA/mm^2		78.8 (8.0)	82.4 (6.8)	76.6 (8.0)	76.6 (8.5)
Inter-group		0.202[b]; 0.023[c]	**0.014**[j]	**0.042**[k]	0.998[l]
Longitudinal BL-FU1		0.059[m]	0.736[m]	**0.007**[m]	**<0.001**[m]
Change rate/year (BL-FU1)		−0.8%	−0.1%	−0.9%	−2.4%
n		43	20	21	49
FU2 UCCA/mm^2		78.3 (8.6)	82.0 (8.1)	76.8 (7.9)	75.7 (10.0)
Inter-group		0.226[b]; 0.149[c]	0.113[j]	0.247[k]	0.894[l]
Longitudinal BL-FU2		**<0.001**[m]	0.055[m]	**0.003**[m]	**<0.001**[m]
Change rate/year (BL-FU2)		−0.8%	−0.9%	−0.9%	−1.2%
Longitudinal FU 1–2		**0.007**[m]	0.273[m]	**0.013**[m]	**0.002**[m]
Change rate/year (FU 1–2)		−0.8%	−0.2%	−0.9%	−0.7%
n		29	12	17	34
BL GM/ml[a]	640.8 (37.7)	647.3 (38.6)	640.1 (43.2)	646.6 (33.4)	644.1 (28.7)
Inter-group	0.705[b]; 0.907[c]	0.748[d]	0.998 [g]	0.939[h]	0.964[e]
		0.828[f]	0.984[j]	0.935[k]	0.989[i]
					0.978[l]
n	34	53	20	21	57
FU1 GM/ml[a]		641.5 (35.1)	636.6 (41.6)	644.6 (31.0)	637.8 (24.9)
Inter-group		0.427[b]; 0.506[c]	0.998[j]	0.789[k]	0.531[l]
Longitudinal BL-FU1		0.510[m]	0.951[m]	0.622[m]	**<0.001**[m]
Change rate/year (BL-FU1)		−0.2%	0.1%	−0.29%	−1.4%
n		40	17	21	43
FU2 GM/ml[a]		637.8 (37.5)	633.5 (48.6)	638.5 (31.6)	630.0 (29.8)
Inter-group		0.358[b]; 0.629[c]	0.934[j]	0.959[k]	0.534[l]
Longitudinal BL-FU2		**0.012**[m]	0.130[m]	0.085[m]	**0.013**[m]
Longitudinal FU 1–2		0.062[m]	0.276[m]	0.128[m]	0.294[m]
Change rate/year (BL-FU2)		−0.7%	−0.7%	−0.7%	−0.7%
n		25	9	15	22
BL WM/ml[a]	528.4 (24.1)	516.8 (28.1)	522.1 (20.0)	509.3 (29.0)	509.3 (25.7)
Inter-group	**0.016**[b]	0.180[d]	0.998[g]	0.172[h]	**0.017**[e]
	0.008[c]	0.415[f]	0.311[j]	0.200[k]	**0.032**[i]
					0.999[l]
n	34	53	20	21	57
FU1 WM/ml[a]		510.8 (24.9)	513.3 (20.4)	508.6 (29.3)	508.7 (23.8)
Inter-group		0.533[b]; 0.536[c]	0.800[j]	0.843[k]	0.996[l]
Longitudinal BL-FU1		0.190[m]	0.084[m]	0.821[m]	0.808[m]
Change rate/year (BL-FU1)		−0.5%	−1.1%	−0.1%	−0.3%
n		40	17	21	43
FU2 WM/ml[a]		503.0 (31.0)	509.3 (26.0)	498.3 (34.6)	507.6 (27.2)
Inter-group		0.915[b]; 0.394[c]	0.985[j]	0.656[k]	0.654[l]
Longitudinal BL-FU2		**0.007**[m]	0.080[m]	**0.043**[m]	**0.001**[m]
Longitudinal FU 1–2		**0.022**[m]	0.455[m]	**0.023**[m]	**<0.001**[m]

Table 2 continued

	Healthy controls	CIS all	CIS non-converting	CIS converting	RRMS
Change rate/year (FU1–2)		−0.7%	−0.4%	−0.9%	−1.3%
Change rate/year (BL-FU2)		−0.7%	−0.9%	−0.6%	−0.6%
n		25	9	15	21

Significance of group differences by univariate ANOVA with post hoc Games–Howell tests for pairwise comparisons (bold: significant with $p < 0.050$)

Subgroups: healthy controls, *CIS all* all baseline CIS patients, *CIS non-converting* baseline CIS patients, who did not convert to MS during follow-up period, *CIS converting* baseline CIS patients who converted to MS during follow-up period, *RRMS* all baseline RRMS patients

[a] Volumes normalized to intracranial cavity volume; change rate/year (%) = mean of volume difference/time difference/initial volume

[b] ANOVA: baseline: controls–all CIS–RRMS, follow-up all CIS–RRMS

[c] ANOVA: controls–CIS non-converting–CIS converting–RRMS; d: post hoc: controls-all CIS

[d] Post hoc: controls–all CIS

[e] Post hoc: controls–RRMS

[f] Post hoc: all CIS–RRMS

[g] Post hoc: controls–CIS non-converting

[h] Post hoc: controls–CIS converting

[i] Post hoc: controls–RRMS

[j] Post hoc: CIS non–converting–RRMS

[k] Post hoc: CIS non–converting–CIS converting

[l] Post hoc: CIS non–converting–RRMS

[m] Significance of longitudinal change rates by paired *t* test (p values)

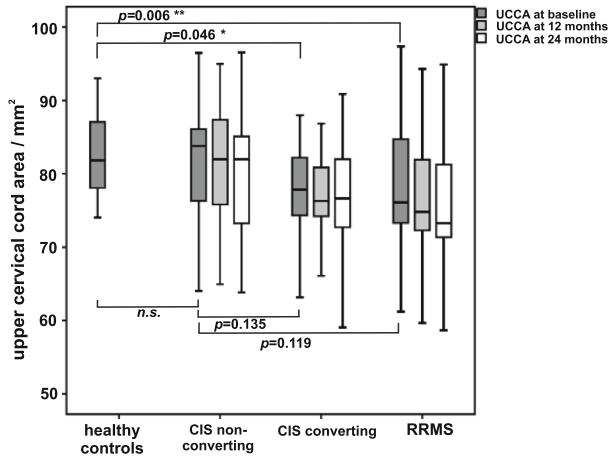

Fig. 1 UCCA in the patient subgroups and in the control group (*boxes* median/interquartile range, *error bars* minimum/maximum). For patients, baseline, follow-up 1 (12 months) and follow-up 2 (24 months) results are presented. Significance of group differences assessed by univariate ANOVA with post hoc Games–Howell tests for pairwise comparisons; significance: $^{**}p < 0.010$, $^{*}p < 0.050$, *n.s.* not significant

For brain GM, significant longitudinal changes were detected during the first year and over 2 years in the RRMS group and over 24 months in the entire CIS group. GM differences between month 12 and 24 were not significant. Annualized atrophy rates of brain GM were lower than SC atrophy rates (all patients: −0.8% during the first year).

Significant correlations of the longitudinal changes of UCCA and brain volumes were detected between the change rates of UCCA (month 12 to month 24) and the change rates of brain GM (BL to month 24, $\rho = 0.453$, $p = 0.001$), but not between changes of UCCA and brain WM.

Effect size and power calculations

The effect sizes (Cohen's *d*) between UCCA in converting CIS and patients non-converting CIS at follow-up, on the basis of mean and SD of the subgroups shown were: baseline $d = 0.550$, month 12: $d = 0.650$, month 2: $d = 0.429$, indicating medium strengths of the effects. In comparison, the discrimination between the two CIS groups by brain WM was considerably weaker (effect size by WM at month 12: $d = 0.186$).

The corresponding power calculation (assumed a type I error rate $\alpha = 0.050$ and a power level of 80%) to estimate the minimum number of patients needed to differentiate between converting CIS and non-converting CIS by cervical cord volumetry at baseline, resulted in $n = 84$ patients.

Impact of cervical cord and brain lesions on spinal and brain volumetry

When comparing patients with or without T1w hypointense cervical cord lesions, we found no significant differences between these groups in terms of baseline UCCA, brain GM or brain WM, either in the whole patient group or within the CIS and RRMS subgroups.

By contrast patients with hyperintense T2 brain lesions had significantly smaller brain GM and WM compared to patients without brain lesions (with/without brain lesions: age-corrected GM 641.8 ± 23.6/624.6 ± 37.1 ml; $p = 0.004$; WM 516.5 ± 22.4/502.0 ± 30.4 ml; $p = 0.015$). No significant differences in UCCA in any patient subgroup, depending on presence of brain lesions were detected.

Associations between cervical cord, brain volumes and clinical disability (Table 3)

At baseline UCCA was significantly negatively correlated with the pyramidal Functional System Score in the entire patient group, indicating that those patients who showed signs of muscular weakness [24% ($n = 26$) who had minimal-to-moderate symptoms relating to pyramidal FSS of 2 or 3 at baseline] had lower UCCA than asymptomatic patients. This correlation was mainly driven by the RRMS subgroup since a similar association was found exclusively in this subgroup.

A significant negative correlation was found between baseline UCCA and EDSS at FU1 in the whole patient group (Fig. 2a).

To further investigate the clinical impact of early SC and brain atrophy concerning the degree of disability, the patients were further categorized according to their EDSS (low degree of disability: EDSS <3; high degree of disability EDSS ≥3). In patients reaching EDSS ≥3 during the follow-up period [$n = 16$ (17%)] UCCA at baseline was significantly smaller than in patients who remained on an EDSS level <3 [$n = 76$ (83%)]; Student's t test: $p = 0.004$). In contrast, brain GM ($p = 0.696$) and WM values ($p = 0.228$) were not significantly different between these EDSS groups.

No significant correlations were detected between the EDSS and brain volumes (age-corrected GM and WM) at baseline or at the follow-up times.

Furthermore, UCCA and the motoric fatigue score were significantly negatively correlated in the whole patient group and in the RRMS subgroup (Fig. 2b), in the sense that at baseline patients who suffered from low-to-severe grade motoric fatigue tended to have lower UCCA than patients without clinically relevant motoric fatigue.

UCCA and brain WM were significantly positive correlated in all patients, in the entire CIS group, converting CIS and RRMS at baseline. The correlation was stronger in CIS patients than in the RRMS group. A significant correlation between UCCA and brain WM was also detected in the healthy control group (correlation coefficient $r = 0.701$, $p < 0.001$) indicating a partly physiological association between cervical cord and brain WM volume [9].

Discussion

The results of this monocentric observational study confirm that upper cervical cord atrophy is present even in the earliest disease stages of MS. Specifically, converting CIS

Table 3 Correlations between UCCA and clinical scores and UCCA and brain WM at baseline (BL) and follow-up (FU1 = 12 months)

	All patients	All CIS	CIS non-converting	CIS converting	RRMS
Pyramidal FSBL vs. UCCA BL[a]	−0.232/0.008*	−0.035/0.805	−0.108/0.325	−0.037/0.437	−0.438/0.001*
	$n = 109$	$n = 52$	$n = 20$	$n = 21$	$n = 57$
EDSS FU1 vs. UCCA BL[a]	−0.225/0.016*	−0.183/0.251	−0.217/0.142	−0.085/0.356	−0.265/0.063
	$n = 91$	$n = 41$	$n = 20$	$n = 21$	$n = 50$
Motoric fatigue BL vs. UCCA BL[a]	−0.253/0.005*	−0.080/0.579	−0.336/0.074	0.141/0.277	−0.323/0.017*
	$n = 104$	$n = 50$	$n = 20$	$n = 20$	$n = 54$
Brain WM BL vs. UCCA BL[b]	0.450/< 0.001*	0.565/< 0.001*	0.523/0.026*	0.682/0.001*	0.353/0.011*
	$n = 108$	$n = 53$	$n = 20$	$n = 21$	$n = 55$

Subgroups: healthy controls, *CIS all* all baseline CIS patients, *CIS non-converting* baseline CIS patients, who did not convert to MS during follow-up period, *CIS converting* baseline CIS patients who converted to MS during follow-up period, *RRMS* all baseline RRMS patients

[a] Spearman correlation ρ/p

[b] Partial correlation corrected for age: r/p

* Significant with $p < 0.050$

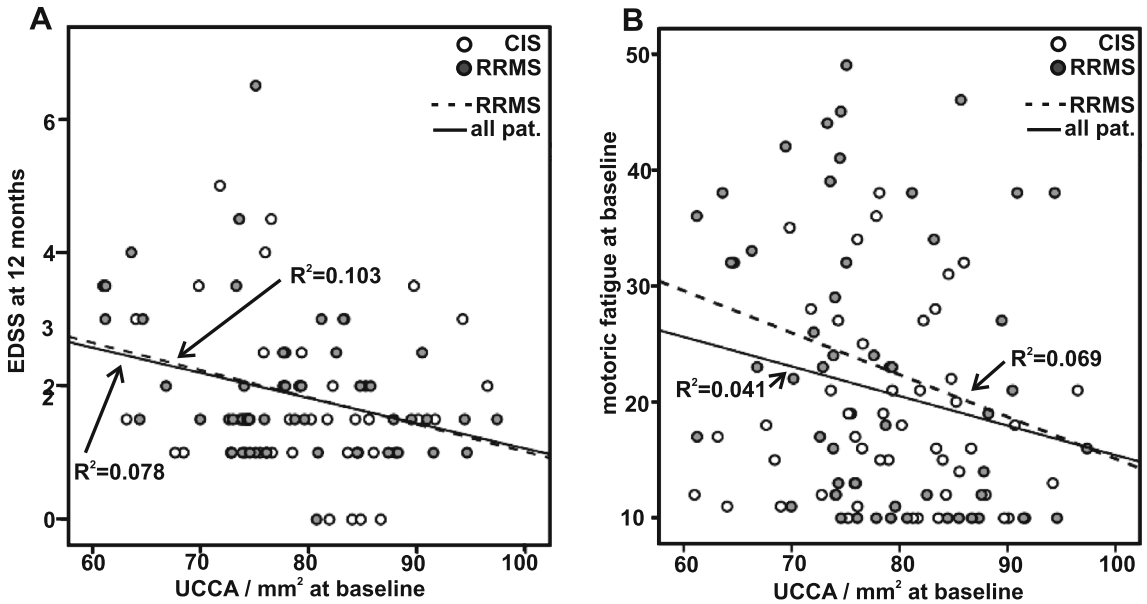

Fig. 2 Associations between baseline UCCA and clinical parameters: **a** association between UCCA at baseline and EDSS at 12 months follow-up; **b** association at baseline between UCCA and motoric fatigue; *dotted lines* linear regression in RRMS (*grey circles*), *solid lines* regression in all patients: RRMS and CIS (*open circles*); R^2 *values* coefficient of determination of the linear regression

patients, but not non-converting CIS patients showed UCCA atrophy at baseline, as well as ongoing spinal cord atrophy during the disease course. Until now, studies including CIS, RRMS patients and HC showed inconsistent results regarding SC atrophy [8, 9, 13]. In line with our study, UCCA atrophy was previously reported in patients with CIS and RRMS, with a higher structural variability in RRMS suggesting occult diffuse neurodegeneration in this MS subtype [9]. In another previous study, SC atrophy in CIS was restricted to patients with baseline brain lesions, but was not found in converting CIS patients [15]. By contrast, including similar numbers of CIS patients, the results of our study did not reveal any significant UCCA differences between CIS patients with and without brain lesions, suggesting SC atrophy as an independent process in the development of the disease. Nevertheless, such differences between different studies might also be attributed to divergent inclusion details, e.g., inclusion of patients with shorter or longer disease durations in the CIS group, or methodological differences.

During follow-up, significant progression of UCCA atrophy was detected in the entire CIS group, converting CIS and RRMS, after the first year and to a lesser extent also after the second year. Such volume decrease over time in CIS patients confirms the results of recent longitudinal findings [18]. Cervical cord atrophy rates were highest in patients with RRMS (up to 2.4% in the first year), which is in line with previous studies reporting similar rates in RRMS [12, 26–28]. Furthermore, converting CIS patients had higher atrophy rates than non-converters. Comparing annualized SC atrophy rates among CIS studies, highest overall atrophy rates of included CIS subjects were found in the present investigations [15, 18]. Including all CIS patients we observed an almost doubled annualized SC atrophy rate (first year 0.8 vs. 0.4%) compared to Brownlee et al., which was mainly driven by CIS patients who converted to MS later on. By contrast, annualized SC atrophy rates in non-converting CIS patients were similar in both studies. The differences between these studies might be attributed to a more rigorous patient selection with a focus on non-spinal cord CIS patients in the Brownlee study. As in our study inclusion criteria were less restricted and dedicated spinal cord imaging has not been performed we cannot fully exclude that our cohort was biased towards a subset of the disease with spinal features. Still, when considering the clinical characteristics of the CIS cohort in the present study such as walking ability, pyramidal, sensory and bladder functional scores and presence of hypointense spinal cord lesions an over-representation of spinal cord affection was not obvious. Nevertheless, other reasons might also contribute in part to this divergent finding including methodological differences in spinal cord volumetry as well as different follow-up periods. Notably annualized UCCA atrophy rates in the study by Brownlee et al. have been calculated on a 5-year follow-up, hence such data represent an averaged rate over a longer period possibly masking different non-linear temporal dynamics that have been described previously for brain [29] and SC atrophy [27]. Specifically, in the current study annualized atrophy rates in the first year were found to be higher

compared to the consecutive time interval. Given this observation one would expect a pseudo-atrophic effect on SC atrophy within the first year after treatment. Although we cannot fully exclude confounding effects due to treatment initiation within the first year, similar distribution of DMT was noted among CIS patients. Hence, 75% of non-converting CIS patients have also received DMT and atrophy rates did not differ between the first and second year in this specific group, suggesting no relevant pseudo-atrophy in the cervical cord overall.

The clinical relevance of early atrophy of the SC and the brain was further investigated by taking the development towards a clinically relevant EDSS threshold into account. Patients reaching EDSS 3 or higher during the follow-up presented significantly smaller baseline UCCA than patients who remained at a lower EDSS level. Neither baseline brain WM nor GM was different between these EDSS groups. This relation was further confirmed by a significant inverse correlation between baseline UCCA and the EDSS after 1 year of follow-up in the entire patient group.

In the analysis of the functional systems this association could be specified by significant inverse correlations of UCCA with muscular weakness (pyramidal functional score) as well as with motor fatigability at baseline. Structural abnormalities in the lateral and posterior columns of the spinal cord containing relevant fiber tracts are likely to contribute to this motor function impairment, as recently shown in relapsing and progressive MS [30, 31]. However, a recent fMRI study could not establish an association between fatigue and structural abnormalities in the spinal cord [32]. Differences in study designs and tests used to assess fatigue probably limit direct comparisons between these studies. Furthermore, as fMRI data were not available in our study, we cannot exclude a possible bias by additional abnormal brain functions on muscular weakness and fatigue.

In line with previous studies, atrophy rates of brain WM and GM differ between CIS and RRMS patients, being higher in RRMS. Further, brain atrophy rates were considerably lower than upper cervical cord atrophy rates [10, 27] and we observed stronger effect sizes for UCCA than for brain atrophy regarding the potential to discriminate between converting CIS patients and patients who remained CIS during follow-up. Reduced brain WM volume was noticed only in RRMS at baseline. Over 24 months of follow-up WM atrophy could be detected in all CIS patients, mainly driven by converting CIS and also in RRMS. In accordance with a recent study [13] WM volume loss was more pronounced in the second year of follow-up in these patients. By contrast, progression of GM volume loss in the first year was restricted to RRMS, while it was detected in all CIS and RRMS during 24 months of follow-up. For RRMS patients our annualized GM atrophy rates are in line with previous findings [33, 34]; however, marked differences were found when focusing on CIS patients. In contrast to [35] we were not able to confirm higher GM atrophy rates in converting compared to in non-converting CIS patients. Again, depending on different follow-up times, atrophy rates differ between these studies, possibly reflecting methodical and technical issues. GM atrophy rates in our study were in line with rates presented by Dalton et al. [32] and Tiberio et al. [33], but consistently higher than in the study by Fisher et al. that used the longest follow-up period to measure GM but also WM atrophy (4 years [34]). As mentioned earlier, averaging atrophy rates over a longer period might possibly mask different non-linear temporal dynamics as recently described for GM [13] and include phases in which atrophy rates may slow down [36, 37]. This hypothesis seems to be supported by the lower and less variable GM and WM atrophy rates in the subgroups when comparing rates of the first year with rates over 24 months.

Apart from this, significant correlations between UCCA and brain WM were found most pronounced in converting CIS and in RRMS suggesting parallel pathological processes in these CNS compartments. This is not contradictory to the above described findings, that UCCA change rates were significant already in the first year while significant brain WM changes were found only in the second year of follow-up. Early volume loss may become evident first in smaller structures, and remain undetectable in the first year in larger structures such as brain WM, especially when using global brain volumetry. The question, to which extent atrophy in the cervical cord is driven by remote pathological processes in the brain, such as secondary Wallerian degeneration, has often been raised. A recent study reported that correlations between atrophy of the cervical cord and brain WM and GM were restricted to early MS, while in longstanding and progressive MS pathological processes in spinal cord and brain seemed to be increasingly decoupled [38]. We hypothesize that, in the situation of first clinical events and early MS, secondary axonal loss driven by the rising remote inflammatory and neurodegenerative cascade in the brain, may accumulate in the small volume of the cervical cord, and could at least in part lead to the herein observed early cervical cord volume loss. Since all descending and ascending projection pathways transect this structure, the clinical eloquence of the upper cervical cord may amplify the effects of the pathological processes commencing in the brain.

Some limitations of our study have to be mentioned. First, although we included more than 100 patients at baseline, not yet all patients had reached the complete follow-up, limiting the power to differentiate between the groups of converting and non-converting CIS. As such, interpretation of our results has to be considered with caution especially for our brain atrophy rates. Nevertheless,

the herein observed conversion rate of CIS patients to CDMS is in line with results from the literature [28]. Second, the follow-up time of 2 years was relatively short, leading to limited progression of EDSS and other clinical parameters. Longer follow-up periods are therefore warranted to study the relevance of spinal cord atrophy in greater detail. Third, we did not further characterize the spatial distribution of WM and GM atrophy in the brain, for example by voxel-based morphometry. Such analyses could provide a higher sensitivity to reflect associations between pathological processes in the brain and spinal cord, and should be part of subsequent investigations.

In conclusion, we confirmed the presence of early cervical cord atrophy in CIS and early MS. Furthermore, cervical cord atrophy may represent a new prognostic marker helping to identify CIS patients at risk of developing MS. These findings strongly support the need of further clinical studies containing cervical cord atrophy as an important measure in early MS.

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Compliance with ethical standards

Ethical standards The study was approved by the local ethics committee (Approval No. 3714-10) and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Written informed consent was obtained from all participants prior to inclusion in the study.

Conflicts of interest BB and AS received funding by the German Federal Ministry for Education and Research, BMBF, German Competence Network Multiple Sclerosis (KKNMS), Grant No. 01GI1601I and Grant No. 01GI0914. CL received a research grant by the German Federal Ministry for Education and Research, BMBF, German Competence Network Multiple Sclerosis (KKNMS), Grant No. 01GI1601I, has received consulting and speaker's honoraria from BiogenIdec, Bayer Schering, Novartis, Sanofi, Genzyme and TEVA, and has received research scientific grant support from Bayer Schering, TEVA and MerckSerono. He holds an endowed professorship supported by the Novartis Foundation. RG received a research grant by the German Federal Ministry for Education and Research, BMBF, German Competence Network Multiple Sclerosis (KKNMS), Grant No. 01GI0914, has received honoraria, consultant fees or other support from Baxter, Bayer Schering, Biogen Idec, CLB, Behring, Genzyme, Merck Serono, Novartis, Talecris, Teva and Wyeth. All other authors declare that they have no conflict of interest in relation to the material presented in this manuscript.

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