Proton Magnetic Resonance Spectroscopy Reveals Central Neuroaxonal Impairment in Systemic Sclerosis

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ABSTRACT. ***Objective***. Involvement of the central nervous system (CNS) in systemic sclerosis (SSc) is rare. Proton magnetic resonance spectroscopy (^{1}H-MRS) assesses *in vivo* cerebral metabolites. We investigated the biochemical modifications of the CNS in SSc.

Methods. N-acetylaspartate/creatine ratio (NAA/Cr) and choline/creatine ratio (Cho/Cr) at right centrum semiovale (RCS) and at right basal ganglia (RBG) were evaluated by ^{1}H-MRS in 12 patients with limited (lSSc) and 8 patients with diffuse SSc (dSSc) and 20 control subjects.

Results. With ^{1}H-MRS, a significant reduction of NAA/Cr ratio at RBG ($p < 0.02$) and at RCS ($p < 0.002$) was detected in SSc patients. Cho/Cr ratio was increased ($p < 0.02$) in the RCS, but not in RBG. In patients with lSSc, a significant reduction of NAA/Cr was detected in RCS but not in RBG.

Conclusion. Evidence of neuroaxonal damage strongly suggests the existence of CNS involvement in SSc. (J Rheumatol 2006;33:546–51)

Key Indexing Terms:
SYSTEMIC SCLEROSIS — PROTON MAGNETIC RESONANCE SPECTROSCOPY
CENTRAL NERVOUS SYSTEM — NEUROMETABOLIC ALTERATIONS

The peripheral nervous system (PNS) is frequently affected by systemic sclerosis (SSc)[1,2], while central nervous system (CNS) involvement is still considered an uncommon complication of the disease; CNS manifestations include migraine, cephalalgia, syncope, extrapyramidal syndrome, generalized seizure, epilepsy, sudden aphonia, hyperreflexia, optic neuropathy, loss of consciousness, progressive confusion, insomnia, depression, psychosis, lethargy, bulbar paralysis, and coma[1].

Electroencephalographic (EEG) modifications described in patients with SSc are generally considered secondary to hypertension and renal involvement[1]. Sympathetic skin responses, consisting of a polysynaptic reflex arch affecting peripheral and CNS control were asymmetric at arms, suggesting a peripheral disturbance in neural control of the reflex arch, probably in association with impaired control at the CNS level. Trigeminal neuralgia is one of the most common early manifestations of nervous system involvement in SSc[1]. Recently, we described blink-reflex alterations (prolonged R2 ipsi- and contralateral latency) in about 15% of asymptomatic SSc patients. This observation has strengthened the hypothesis of damage to the polysynaptic regulatory pathways of the reflex, possibly in the subcortical white matter[3].

Single photon emission computed tomography (SPECT) demonstrated a focal, mild cerebral perfusion defect, worsened by cold test, in 3/9 SSc patients[4].

Proton magnetic resonance spectroscopy (^{1}H-MRS) reveals the functional status of cerebral areas, evaluating *in vivo* the presence of N-acetylaspartate (NAA), choline (Cho), and creatine (Cr) metabolite ratios in voxel units (graphic information that defines a point in 3-dimensional space). Thus, ^{1}H-MRS acquires a signal from rapid changes in radio frequencies, indicative of the free concentration of biochemical compounds[5]. ^{1}H-MRS can evaluate neuronal markers, energy consumption, tissue metabolism, maturation, and grade of myelination of white matter in the brain[5].

Our aim was to study CNS damage in SSc by investigating the biochemical modifications of the CNS in SSc, evaluating cerebral metabolite ratios through ^{1}H-MRS.

MATERIALS AND METHODS

Patients and controls. Twenty patients (19 female, one male) with SSc were recruited at the Department of Medicine, Section of Rheumatology, of the University of Florence. They were classified as limited (lSSc; 12 patients) and diffuse (dSSc; 8 patients) subsets of the disease, on the basis of the extent of

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Accepted for publication October 19, 2005.

skin involvement[6]. Patient characteristics are shown in Table 1. Twenty healthy subjects, matched for sex and age (19 females, one male; mean age 46.9 ± 8.4 yrs) served as controls.

All subjects underwent a clinical-hematological assessment[7]. The following investigations were performed: neurological examination and psychiatric consultation; nailfold videocapillaroscopy; upper and lower limb electromyography (EMG); EEG; visual evoked potentials (VEP), magnetic resonance imaging (MRI), and ^{1}H-MRS; hematic concentrations of folates and cobalamine; antinuclear autoantibodies, anti-DNA, anticentromere, extractable nuclear antigen including antitopoisomerase I (Scl-70), Ro/SSA, La/SSB, and others; rheumatoid factor, anticardiolipin, anti-ß$_2$-glycoprotein I, and lupus anticoagulant (LAC).

Nailfold videocapillaroscopic pattern was classified as early, active, or late[8]. Declaration of Helsinki statements were respected. All subjects were fully informed about the purpose of the study and gave written consent to participate. Exclusion criteria both for SSc and controls were: acute or chronic concomitant pathologic conditions (diabetes mellitus, lung emphysema, blood hypertension, vasculitides, enteric malabsorption, reduced folate or cobalamine serum levels), PNS and CNS involvement symptoms, tobacco smoking, treatment by drugs interfering with PNS and CNS (α/ß-agonist/antagonist, parasympathetic stimulatory or inhibitory drugs, antidepressants, antipsychotics, and psychotropic substances), and, for SSc patients, other associated immunologic diseases, the impossibility of a 15 day washout for the presence of severe cutaneous ulcers, pulmonary hypertension, alveoli-

Table 1. Characteristics of patients. Values are number of patients unless otherwise indicated.

	SSc	ISSc	dSSc	Controls	p
Sex					
Male	1	0	1	1	
Female	19	12	7	19	
Mean age, yrs, mean ± SD	51.5 ± 12.2	50.6 ± 12.2	52.9 ± 12.9	46.9 ± 8.4	
Subtype of disease	—	12	8	—	
Mean disease duration*, yrs, mean ± SD	3.6 ± 2.8	4.42 ± 3.0	2.38 ± 2.1	—	
Immunological findings†					
ANA (+)	18	12	8	0	
Scl-70 (+)	8	0	8	0	
ACA (+)	12	11	0	0	
La/SSB (+)	2	2	2	0	
Serum concentration‡, mean ± SD					
Folates	9.7 ± 4.51	10.5 ± 4.22	8.4 ± 4.92	9.1 ± 4.37	
Vitamin B12	576.3 ± 148.04	579.8 ± 172.04	562.6 ± 213.97	578 ± 34.21	
Videocapillaroscopic pattern					
Early	5	4	1	0	
Active	10	6	4	0	
Late	5	2	3	0	
Neurologic symptoms					
Migraine	1	0	1	2	
Tension-type cephalalgia	6	4	2	5	
Epilepsy	0	0	0	0	
TIA/stroke	0	0	0	0	
Anxiety disorder	5	3	2	1	
Depressive syndrome	3	1	2	1	
Psychosis	0	0	0	0	
Carpal tunnel syndrome	3	1	2	0	
Trigeminal neuropathy	2	1	1	0	
Other sensorimotor peripheral neuropathies	0	0	0	0	
EEG abnormalities	0	0	0	0	
VEP abnormalities	0	0	0	0	
^{1}H-MRS findings, mean ± SD					
RBG (NAA/Cr)	2.20 ± 0.22 (*)	2.20 ± 0.20	2.19 ± 0.25	2.33 ± 0.10 (*)	0.02
RBG (Cho/Cr)	1.31 ± 0.09	1.31 ± 0.07	1.31 ± 0.12	1.27 ± 0.03	
RCS (NAA/Cr)	2.46 ± 0.21 (*)	2.45 ± 0.20 (**)	2.46 ± 0.24	2.63 ± 0.07 (*) (**)	0.002 0.02
RCS (Cho/Cr)	1.30 ± 0.08 (*)	1.29 ± 0.10	1.30 ± 0.06	1.25 ± 0.03 (*)	0.02

* Onset of disease calculated as years from date of diagnosis (not from Raynaud's phenomenon starting). Cutoff between early and late phases of disease 10 years for ISSc and 5 years for dSSc. † Rheumatoid factor, lupus anticoagulant, anticardiolipin, ß$_2$ glycoprotein I, anti-DNA, and Ro/SSA were normal in all patients and controls (data not shown). ‡ Normal range of folates and vitamin B12 serum concentrations, given by our Radiometric Analysis Laboratory, are respectively, 10 ± 7 ng/ml and 575 ± 395 pg/ml. * ^{1} H-MRS values significant by t test. ** ^{1}H-MRS values significant by Scheffé test. ANA: antinuclear antibody, ACA: anticentomere antibody, TIA: transient ischemic attack.

tis, renal failure, severe cardiac or gastrointestinal involvement, and previous potential neurotoxic therapy. Before ^{1}H-MRS evaluation, patients underwent a 15 day pharmacological washout and only proton pump inhibitors (15 patients), H2-blockers (14 patients), and sucralfate (10 patients) were allowed. For control subjects, any kind of drug was strictly forbidden for 15 days before ^{1}H-MRS.

MRI and ^{1}H-MRS analysis. All subjects underwent diagnostic MRI and ^{1}H-MRS with a 1.5 T apparatus (Eclipse, Philips, The Netherlands) operating at 27mT/m gradient strength and 75mT/m/ms slew rate using a circularly polarized head coil. The standard MRI examination included: sagittal spin-echo T1-weighted sequence (TR 441 ms, TE 12 ms, 240 mm field of view, 6 mm slice thickness, gap 1.0, NSA 2); transverse double spin-echo T2-weighted sequence (TR 3000 ms, TE 12/96 ms, 240 mm FOV, 5 mm slice thickness, gap 1.0, NSA 2); transverse fluid attenuated inversion recovery (FLAIR) sequence (TR 6000 ms, TE 96 ms, TI 1800 ms, 240 mm FOV, 5 mm slice thickness, gap 1.0, number of signal averages 1); and coronal turbo T2-weighted sequence (TR 17299 ms, TE 84 ms, 240 mm FOV, 5 mm slice thickness, gap 1.0, NSA 2). For ^{1}H-MRS, single-voxel acquisition was obtained using a 900-1800-1800 pulse sequence (PRESS, TR = 1500 ms, TE = 270/40 ms, double-echo acquisition) and post-processed as described[9]. A volume of interest (VOI) of 20 × 20 × 20 mm was positioned on the right centrum semiovale (RCS), thus including mainly white matter, and a similar voxel was positioned on right basal ganglia (RBG). Before the acquisition of localized proton MR images of brain metabolites, the brain water proton signal was suppressed by excitation pulse angle (CHESS technique). The total duration of MRI and ^{1}H-MRS was about 50 minutes. Values were normalized to creatine resonance intensity so that results were expressed as ratio to creatine. We assessed the 2 main metabolite ratios of N-acetylaspartate/creatine (NAA/Cr) and choline/creatine (Cho/Cr) at RBG and at RCS in the white matter.

Assessment for presence of brain involution was based on evaluation of the widening of sulci and narrowing of gyri on T1-weighted MRI sequences, scoring the findings from 0 (no brain involution) to 1 (brain involution).

An expert radiologist (MM) read the brain images, prepared the data, and did the statistical analysis.

Statistical analysis. All ^{1}H-MRS data were expressed as mean ± standard deviation (SD). Comparisons between controls and SSc patients were performed by 2-tailed t test for unpaired data (p values < 0.05 were considered significant). One-way ANOVA and post-hoc Scheffé test were used to compare ^{1}H-MRS result means among controls, lSSc, and dSSc. Chi-square was used to find differences between controls and SSc in frequency of brain lesion or brain involution. Pearson's correlation analysis was used to study significant correlations between ^{1}H-MRS parameters and age of the patients, disease duration, videocapillaroscopy result, and autoantibody pattern (significant if $p < 0.01$), for neurological symptoms versus MRI findings and versus ^{1}H-MRS results.

RESULTS

MRI detected brain lesions in white matter in 70% of patients and in 25% of controls (chi-square: $p < 0.005$; OR = 0.142). Lesions were more frequent in lSSc (75%) (Figure 1A) than in dSSc patients (62.5%). Signs of brain involution were found in 40% of patients (Figure 1B) and in 35% of controls. ^{1}H-MRS spectra of SSc patients had slightly decreased NAA/Cr and increased Cho/Cr ratio in respect to controls (Figure 2). Moreover (Figure 3 and Table 1), NAA/Cr was significantly lower (2-tailed t test) in SSc than in controls both at RBG (2.20 ± 0.22 vs 2.33 ± 0.10; $p = 0.02$) and at RCS (2.46 ± 0.21 vs 2.63 ± 0.07; $p = 0.002$). Cho/Cr was significantly higher in SSc than in controls at RCS (1.30 ± 0.83 vs 1.25 ± 0.28; $p = 0.02$), while at RBG it was not different from controls. No significant differences were detected between lSSc and dSSc. The analysis of the 3 groups (controls, lSSc, dSSc) by one-way ANOVA with the post-hoc Scheffé test demonstrated a significant difference of NAA/Cr content at RCS in lSSc versus controls ($p = 0.02$) and a tendency in dSSc versus controls that did not reach significance. Other ^{1}H-MRS parameters were not significantly different. No correlation between ^{1}H-MRS and age of patients, disease duration, autoantibodies, capillaroscopic patterns, or presence of brain lesions or of brain involution was observed. Moreover, upper and lower limb EMG, EEG, and VEP showed normal results in our group. Tension-type cephalalgia, carpal tunnel syndrome, trigeminal neuropathy, migraine, anxiety disorder, and depressive syndrome were the neurological symptoms more frequently found in our patients: no correlations were found among neurological symptoms and MRI abnormalities and ^{1}H-MRS results.

DISCUSSION

CNS involvement in SSc is considered rare, due to the scarcity of brain connective tissue[10], which is localized mainly in basement membrane of cerebral vessels and in leptomeninges. Alternatively, CNS symptoms may be found in advanced SSc, but secondary to atherosclerosis of cerebral vessels, chronic hypertension, or damage induced by anticardiolipin antibodies[1,10]. Nevertheless, psychiatric manifestations may be an early clinical feature of SSc[11], suggesting primary CNS involvement.

To our knowledge, this is the first study that investigated a direct involvement of CNS in SSc by ^{1}H-MRS, an unconventional technique, in the absence of other confounding factors such as arterial hypertension, anticardiolipin antibodies, vitamin B12 and/or folate deficiency, or drug-induced neurotoxicity (i.e., cyclophosphamide).

Neuroimaging evaluation. Previous studies by computerized tomography identified brain involution in an elevated percentage of SSc patients[11]. The similar incidence of widening of sulci and narrowing of the gyri in SSc versus controls (40% vs 35%) indicates that brain involution is not more frequent in SSc than in a normal population.

Our cohort of patients with SSc showed a scarcity of central neurological symptoms/signs. Only anxiety and major depression were relatively frequent in our patients.

In accord with recent studies[12], the MRI study we performed showed an elevated incidence in SSc patients of subcortical hyperintense lesions, mainly localized in white matter. MRI or ^{1}H-MRS alterations did not correlate with neurological manifestations.

Although our work does not permit confirmation of this hypothesis, the white matter lesions could be due to an ischemic mechanism, so that cerebral vasospastic events may be involved in the genesis of the brain lesions we found[9]. According to this hypothesis, a reduced cerebrovascular reserve, or even microvascular damage of the CNS due to Raynaud's phenomenon, was described in SSc[4]. Further, a

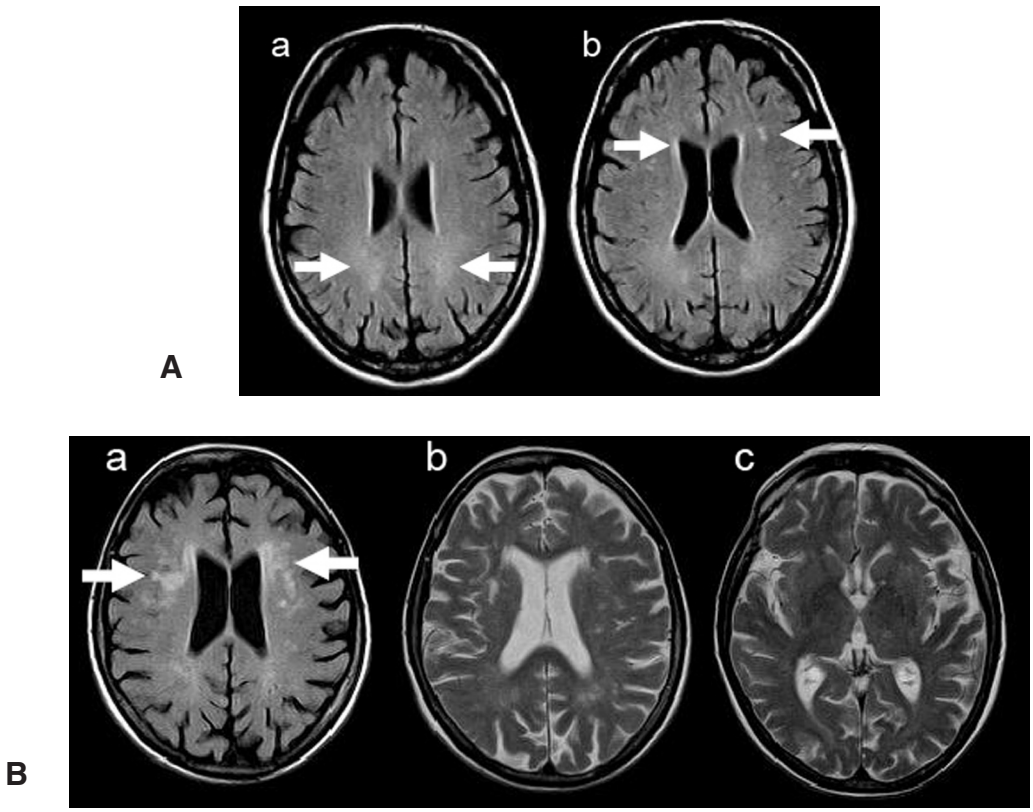

Figure 1. A. MR axial image in a 33-year-old woman with lSSc: the 2 axial FLAIR sequences (a, b) show scattered hyperintense lesions (arrows) localized on periventricular and subcortical white matter. B. MR axial image in a 72-year-old woman with dSSc: axial T2-weighted (a) and FLAIR (b, c) images show multiple hyperintense lesions (arrows) and enlargement of ventricles and supratentorial subarachnoid spaces.

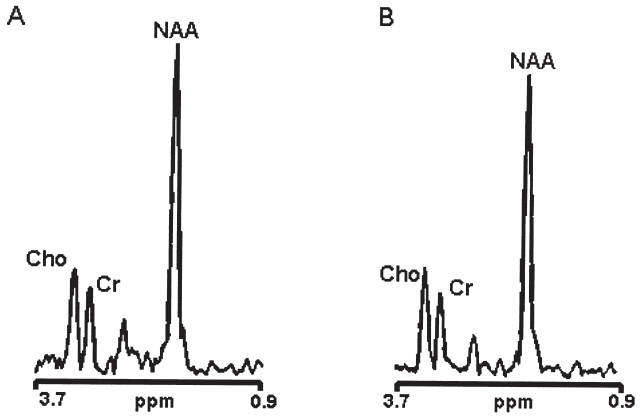

Figure 2. ^{1}H-MRS spectra in the healthy control group (A) and in the SSc patient group (B). The SSc group presents slightly decreased N-acetylaspartate/creatine (NAA/Cr) and increased choline/creatine (Cho/Cr) ratios.

recent study detected a regional cerebral blood flow reduction in SSc by transcranial Doppler sonography using the ^{133}xenon-clearance technique, more frequently in patients with late capillaroscopic alterations[13], suggesting a correlation between cerebral hypoperfusion and sclerodermic microangiopathy.

The above hypothesis, however, is not unanimously supported. We did not find a relationship between neuroimaging (and ^{1}H-MRS) and microvascular alterations (studied by nailfold capillaroscopy). Another study showed cerebral hypoperfusion by SPECT in more than 50% of SSc patients, and MRI alterations in 57% of patients positive and in 25% negative for hypoperfusion, without correlation with capillaroscopic pattern[14]. A suggestive, but hardly supportable interpretation of these findings is the potential independence of the neurological from vascular injury. Moreover, the reason for the absence of abnormalities on EEG and VEP, both sensitive techniques, in patients that showed MRI alterations is unclear. Possibly the number, extent, and localization of the cerebral white matter lesions are insufficient to determine brain electrical activity modifications. On the other hand, the lack of a controlled analysis of the MRI images by more than one radiologist could be a cause of bias.

Spectroscopic evaluation. NAA is a metabolite contained almost exclusively in neurons and neuronal processes in mature brain; therefore it is used as marker of axonal integrity and density. ^{1}H-MRS analysis demonstrated a diffuse decreased NAA/Cr ratio in our patients. This reduction may be interpreted as an index of neuroaxonal damage/loss and gliosis, as clearly demonstrated in other disorders[15], usually due to recurring cerebral ischemic events.

Moreover, the elevated Cho/Cr ratio we found in the white

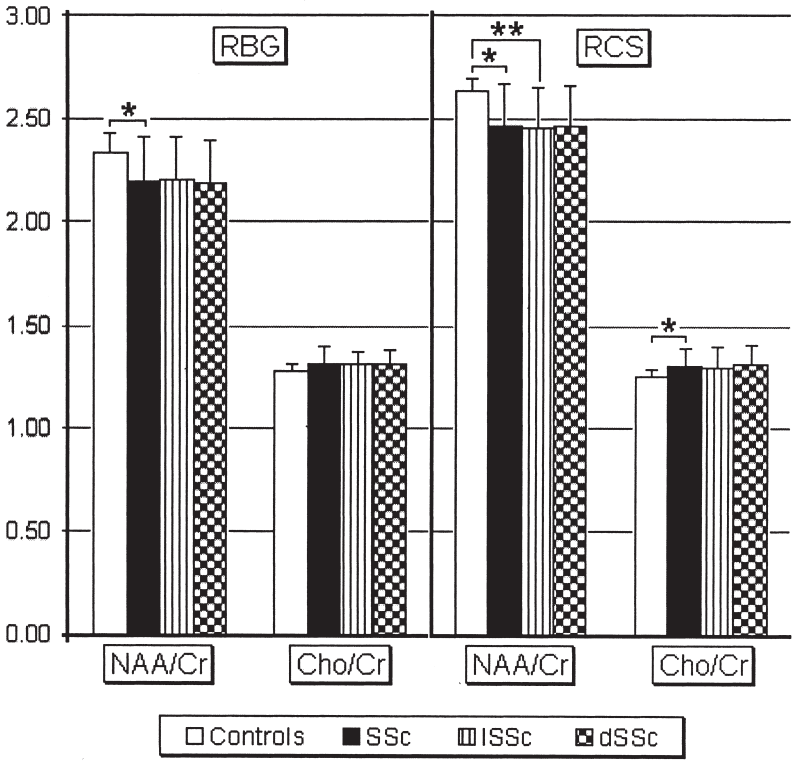

Figure 3. Mean (± SD) metabolite ratios in controls (white bars) and all SSc patients as a group (black bars), lSSc (striped bars), and dSSc (patterned bars). N-acetylaspartate/creatine (NAA/Cr) and choline/creatine (Cho/Cr) ratio values are shown, assessed at right basal ganglia (RBG) and right centrum semiovale (RCS). Statistically significant differences ($0.05 > p > 0.001$) by *2-tailed t test; **one-way ANOVA with post hoc Scheffé test.

matter indicates an increased turnover of the neuronal cellular membrane, because choline is a marker of the metabolism of its constituents (glycosphingolipids). These data, in association with the increased T2 signal in MRI images, suggest that an inflammatory/demyelination event, as reported in other pathological conditions, could contribute to neuroaxonal damage in SSc: an increased Cho/Cr ratio was described in normal-appearing white matter of patients with multiple sclerosis, indicating the existence of a "pre-lesional myelin membrane" pathology[16]. But once again we are not able to confirm the validity of this hypothesis in patients with SSc.

Finally, cerebral neurometabolic modifications were more prominent in lSSc than in dSSc. Although an association between disease duration and ^{1}H-MRS parameters was not found, the longstanding disease duration in lSSc patients (Table 1) could partially explain our findings, as highlighted also by the major number of lesions retrieved in this group.

Our data suggest that ^{1}H-MRS reveals neurometabolic involvement of the CNS, mainly at the white matter, and that the axonal integrity and density is disrupted in patients with SSc. This could be due to microvascular damage and/or to inflammatory/demyelination status.

These findings do not correlate with clinical symptoms, laboratory data, videocapillaroscopic pattern, EEG or VEP studies, or worse CNS neuroimaging status. This suggests that the neuroaxonal disorder we found in SSc is usually limited or has a localization that is not significant, and, perhaps, is very slowly progressive. Further studies in a larger number of patients are required to validate these preliminary data.

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