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Motor unit number index (MUNIX) in the quantitative assessment of severity and surgical outcome in cervical spondylotic amyotrophy

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HIGHLIGHTS

- The MUNIX technique can detect motor unit loss in cervical spondylotic amyotrophy (CSA), even in the pre-symptomatic stage.
- MUNIX is more sensitive to motor unit loss in CSA compared to motor functional measures and CMAP.
- MUNIX may serve as a supplementary test to routine EMG to quantitatively evaluate CSA.

ABSTRACT

Objective: To assess the feasibility of motor unit number index (MUNIX) in the quantitative assessment of the cervical spondylotic amyotrophy (CSA).
Methods: MUNIX was recorded bilaterally on the abductor pollicis brevis, abductor digiti minimi, biceps brachii and middle deltoid in 41 normal controls and 47 patients with CSA (distal-type to proximal-type ratio: 25 to 22). Additionally, patients were assessed on handgrip strength (HGS), the disabilities of arm, shoulder and hand (DASH) and Medical Research Council (MRC) scales. These examinations were re-evaluated approximately 18 months after surgery in 37 of these CSA patients.
Results: MUNIX values were noticeably lower in the mainly affected muscles of CSA patients than those in controls ($P < 0.05$), and 49.0% (51/104) of the tested muscles with abnormal MUNIX measurements showed normal muscle strength. Significant correlations between MUNIX measurements and both DASH and MRC scores were observed in both CSA patient groups ($P < 0.05$). Postoperative longitudinal follow-up analysis identified significant increase in motor unit number in both CSA patient groups within approximately 18 months ($P < 0.05$), with or without improved measures of motor function.
Conclusions: A significant reduction in MUNIX values related to motor impairment was observed in CSA patients, even in the subclinical stage. Compared to measures of motor function, the MUNIX measurements in the patients with CSA improved more noticeably after surgical intervention.
Significance: MUNIX may serve as an available supplementary test to quantitatively evaluate the motor dysfunction in CSA and to track its progression, that is complementary to conventional electromyography.

1. Introduction

Cervical spondylotic amyotrophy (CSA), an uncommon cervical spondylosis, is always characterized by muscle atrophy and weakness of upper limbs without marked sensory disturbance or lower limb involvement (Keegan, 1965; Shinomiya et al., 1994).

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According to the most severely involved muscle, CSA is generally divided into distal and proximal types (Shinomiya et al., 1994).

The pathogenic mechanism of CSA is unclear. The current hypotheses mainly implicate selective injury of the spinal anterior roots and/or anterior horn caused by direct compression and/or indirect circulatory insufficiency (Keegan, 1965; Yanagi et al., 1976; Kameyama et al., 1998). Thus, some doctors tried to do cervical decompression procedures in patients with CSA to eliminate compression of nerves and/or blood vessels (Fujiwara et al., 2006; Uchida et al., 2009; Inui et al., 2011; Imajo et al., 2012; Wang et al., 2014). In all of these studies, both disease progression and surgical outcome in CSA are typically assessed by functional rating scales and/or scoring systems (Fujiwara et al., 2006; Uchida et al., 2009; Inui et al., 2011; Imajo et al., 2012; Wang et al., 2014). However, these clinical assessments suffer from great variability and are suboptimal to observe subtle changes in disease advance or response to the treatment (Fatehi et al., 2017; Philibert et al., 2017; Querin et al., 2018). Furthermore, although conventional electrophysiological examinations and imaging evaluations are important parts of the diagnosis of CSA (Hatanaka et al., 2017; Zheng et al., 2017a), these clinical tools are rarely used for the follow-up of CSA since they are less sensitive and less relevant in assessing both disease severity and its advance (Neuwirth et al., 2017; Jacobsen et al., 2019).

The motor unit number index (MUNIX) is a novel quantitative method that provides an index of the number and size of the functional motor unit in the tested muscles rather than absolute values (Nandedkar et al., 2010). Compared with the incremental, multiple point and F-wave motor unit number estimation (MUNE) procedures (McComas et al., 1971; Wang and Delwaide, 1995; Shefner et al., 2011; de Carvalho et al., 2018), MUNIX can be performed in proximal muscles in which a maximal CMAP can be elicited. Furthermore, the calculation of MUNIX mainly relies on the negative amplitude of the compound muscle action potential (CMAP) and the surface electromyography (EMG) interference pattern (SIP) obtained by varying levels of isometric contraction (Nandedkar et al., 2010). Therefore, MUNIX included both main types (type-I and type-II) of motor units, while spike-triggered MUNE can only selectively detects type-I motor units (de Carvalho et al., 2018). Compared with the quantitative motor unit potential (MUP) analysis that mainly evaluate the measurements (e.g., mean area, amplitude and duration) of MUP (Jacobsen et al., 2018), MUNIX can provide an estimated index of functional motor unit number in tested muscles. Therefore, MUNIX is considered to be useful in some disorders in which the motor units are quickly lost or temporarily suppressed without compensatory reinnervation compared to the quantitative MUP analysis (de Carvalho et al., 2018). More importantly, unlike spike-triggered MUNE method and quantitative MUP analysis, MUNIX is a non-invasive technique that requires only a small amount of electrical stimulation, thus facilitating patient compliance so that they do not be afraid for the follow-up studies.

Recently published reports demonstrated the value of MUNIX in the quantitative assessment of disease severity and in the longitudinal follow-up of motor unit loss in many different neuromuscular diseases, such as adult spinal muscular atrophy (SMA) (Querin et al., 2018), amyotrophic lateral sclerosis (ALS) (Nandedkar et al., 2010; Boekestein et al., 2012; Neuwirth et al., 2017), anti-MAG neuropathy (Fatehi et al., 2017), chronic inflammatory demyelinating polyradiculoneuropathy (Delmont et al., 2016) and Charcot-Marie-Tooth disease (CMT) (Bas et al., 2018). However, few studies involving the quantitative evaluation of CSA by MUNIX have been reported, although it may guide the treatment of CSA.

The present study aimed to quantify functional motor units in both distal- and proximal-type CSA patients and analyse the correlation between MUNIX and clinical functional measures in both CSA patient groups. In addition, we analysed the change of MUNIX in the patients with CSA after cervical decompression.

2. Materials and methods

2.1. Subjects

Forty-seven patients with CSA (distal-type to proximal-type ratio: 25 to 22) and 41 healthy subjects were included in the present study (Supplementary Table 1). All subjects in both CSA patient groups were recruited in Huashan Hospital from October 2015 to December 2017. The study protocol was approved by Human Ethics Committees (Huashan hospital, Fudan University, China). All subjects gave informed consent.

The subjects in normal control and CSA patient groups were chosen based on the inclusion and exclusion criteria published previously (Zheng et al., 2017a). All patients with CSA were followed-up for more than six months after first visit to identify the diagnosis, and the patients would be excluded from the present study when they were suspected to have an alternative diagnosis during the follow-up. All patients with identified CSA underwent anterior cervical decompression and fusion with internal fixation (ACDF) by the same senior surgeon, and detailed surgical procedures have been reported in our previous study (Wang et al., 2014). The detailed surgical segments for each patient are listed in Supplementary Table 2.

2.2. Testing methods

2.2.1. Motor unit number index

The MUNIX reported by Nandedkar et al. was applied in all 41 normal controls and 47 patients with CSA before operation (Nandedkar et al., 2010), and 37 of these CSA patients (distal-type to proximal-type ratio: 18 to 19) underwent repeated MUNIX examination approximately 18 months after surgery.

The maximal CMAP was recorded bilaterally from the abductor digiti minimi (ADM), abductor pollicis brevis (APB), middle deltoid (Del) and biceps brachii (BB) in a belly-tendon montage (filters: 3 Hz–10 kHz) to supramaximal stimulation. In this step, several position of the active electrode will be tested to obtain maximal CMAP amplitude. Subsequently, two series of SIPs were recorded in a 300-ms window. Each recording was performed with the subject instructed to maintain an isometric contraction against the manual resistance at five different force levels (10% or slight, 25%, 50%, submaximal, and maximal contraction) (filters: 10 Hz–1000 Hz).

According to the raw measurements of both SIP and CMAP, MUNIX values for each muscle were calculated using a dedicated analysis Excel table. The motor unit size index (MUSIX) was then measured using the following formula: MUSIX = baseline-to-peak amplitude of maximal CMAP/MUNIX. Furthermore, MUNIX sum-scores and CMAP sum-scores were measured based on the summation of these scores of all tested muscles, respectively. Of all tested muscles, respectively. To avoid the influence of volume conduction from other muscles, MUNIX was not be calculated when one of the following conditions occurred: SIP area less than 20 mV ms, ideal case motor unit count (ICMUC) greater than 100, SIP area/CMAP area less than 1, or CMAP amplitude less than 0.5 mV (Nandedkar et al., 2010).

For the evaluation of intra-rater reproducibility, MUNIX values of 15 normal controls (right side) and 25 CSA patients (distal-type to proximal-type ratio: 11 to 14) (symptomatic side) were tested twice by same examiner in more than 30 min intervals at the initial assessment, with electrodes being completely removed and repositioned for the second test. As we did in the first test, the CMAP amplitudes must be maximized by moving the electrodes in the second test.

All electrophysiological examinations were carried out by Keypoint EMG machine (version 2.32; Medtronic Dantec, Skovlunde, Denmark), and all tests were performed at a skin temperature >32 °C. To exclude the influence of inter-rater variability, all MUNIX tests were performed by the same examiner.

2.2.2. Clinical functional, imaging and needle EMG evaluation

All patients with CSA underwent muscle strength examination, handgrip strength (HGS) test, disabilities of the arm, shoulder and hand (DASH) measures, needle EMG detection and cervical magnetic resonance imaging (MRI) evaluation before operation, and all of these tests were re-evaluated approximately 18 months after surgery in 37 of these CSA patients (distal-type to proximal-type ratio: 18 to 19).

Muscle strength was summarized as the Medical Research Council (MRC) score by manual testing in each tested muscle. MRC sum-scores were measured based on the summation of MRC scores of all tested muscles. According to the protocol reported previously (Zheng et al., 2017b), bilateral HGS tests were performed using a Jamar hydraulic hand dynamometer (Sammons Preston Rolyan, Illinois, USA). Needle EMG was performed to identify the bilateral or unilateral upper limb involvements before operation, and this detection was also used to reveal the number of the CSA patients who presented with motor unit action potential (MUAP) changes and/or spontaneous activities (i.e., fibrillation potentials, positive sharp waves, and complex repetitive discharges) in at least one of the tested muscles before and after surgery.

In both CSA patient groups, parameters were considered abnormal if these parameters were absent; if MUSIX values were 2 standard deviations (SDs) more than the mean value of healthy subjects; or if CMAP amplitude, MUNIX values or HGS were 2 SDs less than the mean values of healthy subjects.

2.3. Statistical methods

All measurements were evaluated by SPSS version 12.0 (IBM, USA). Normally distributed data was tested by the Kolmogorov-Smirnov test. Parameters among distal-type CSA patients, proximal-type CSA patients and normal controls were analysed using one-way ANOVA (Bonferroni correction) or Kruskal-Wallis H test. The frequencies of abnormal MUNIX measurements, imaging abnormalities, needle EMG findings and clinical symptoms between two CSA patient groups were analysed by Fisher's exact test, and the same tests were used to analyze the frequencies of absent spontaneous activities between the initial and follow-up assessments. Pearson or Spearman correlation coefficient analysis (CCA) was used to evaluate the relationship between each parameter of MUNIX tests and both clinical functional measures and disease duration. The test-retest reproducibility of MUNIX in normal controls and patients with CSA were analysed using both interclass correlation coefficient (ICC) methods, CCA, and coefficient of variation (COV). The COV was measured by the formula reported previously (Nandedkar et al., 2010). In the follow-up group of the patients with CSA, the parameters between preoperative and postoperative assessments were analysed using the paired t-test or Wilcoxon signed rank test. A *P*-value less than 0.05 was considered significant.

3. Results

3.1. Preoperative/Initial assessment

The measurements of both MUNIX tests and clinical functional measure in both control and CSA patient groups are listed in Tables 1 and 2. Furthermore, the test-retest reproducibility of all MUNIX measurements (e.g., CMAP amplitudes, MUNIX values and MUSIX values) in each tested muscle in both control and CSA patient groups are presented in the Supplementary Table 3, along with the correlation between the clinical functional measures and these MUNIX measurements in the Supplementary Table 4.

3.1.1. Measurements between control and patient groups

In the proximal-type CSA patient group, a significant reduction in MUNIX values, as well as increased MUSIX values, were observed in both the Del and BB on the symptomatic side compared with normal controls ($P < 0.05$, Table 1). These patients also presented with reduced CMAP amplitudes and MRC scores in both the Del and BB on the symptomatic side ($P < 0.05$, Table 1).

In the distal-type CSA patient group, both reduced MUNIX values and decreased CMAP amplitudes were found in both symptomatic-side APB and symptomatic-side ADM when compared to normal controls, whereas MUSIX values in both the APB and ADM increased significantly on the bilateral side ($P < 0.05$, Table 2). Furthermore, these patients presented with reduced MRC scores in both the APB and ADM on the bilateral side, while symptomatic-side HGS was obviously lower compared to normal controls ($P < 0.05$, Table 2).

In both distal- and proximal-type CSA patient groups, MUNIX sum-scores, CMAP sum-scores and MRC sum-scores were all obviously lower than those of normal controls ($P < 0.05$, Tables 1 and 2).

3.1.2. Reproducibility in both CSA patient and control groups

A strong positive correlation of all measurements of MUNIX tests between repeated tests in the initial assessment was observed in each tested muscle in both the patients with CSA and healthy subjects ($P < 0.05$, Supplementary Table 3). Both ICC and VOC values further identified good reproducibility of all MUNIX measurements in both the patients with CSA and healthy subjects (Supplementary Table 3).

3.1.3. Correlation between MUNIX and motor functional measures

In patients with proximal-type CSA, significant negative correlations were observed between DASH and both MUNIX sum-scores ($r = -0.51$; $P < 0.05$) and MUNIX values on the symptomatic side (Del: $r = -0.66$; BB: $r = -0.57$; $P < 0.05$) (Supplementary Table 4), and MRC scores were positively correlated with both MUNIX sum-scores ($r = 0.64$; $P < 0.05$) and MUNIX values on the symptomatic side (Del: $r = 0.84$; BB: $r = 0.92$; $P < 0.05$) (Supplementary Table 4).

In patients with distal-type CSA, there were negative correlations between DASH and both MUNIX sum-scores ($r = -0.70$, $P < 0.05$) and MUNIX values in the bilateral ADM ($r = -0.50$ and -0.64, $P < 0.05$), APB on the symptomatic side ($r = -0.46$, $P < 0.05$) and bilateral Del ($r = -0.41$ and -0.47, $P < 0.05$) (Supplementary Table 4). In addition, a positive correlation between HGS and MUNIX values was observed in the bilateral APB ($r = 0.77$ and $r = 0.53$, $P < 0.05$) (Supplementary Table 4), and MRC scores were significantly correlated with both MUNIX sum-scores ($r = 0.76$, $P < 0.05$) and MUNIX values in the bilateral ADM ($r = 0.80$ and 0.69, $P < 0.05$), bilateral APB ($r = 0.80$ and 0.46, $P < 0.05$) and BB on the symptomatic side ($r = 0.51$, $P < 0.05$) (Supplementary Table 4).

3.2. Postoperative/Second assessment

Postoperative imaging evaluation demonstrated that both compression and stenosis, as well as cervical kyphosis, were corrected at the surgical segments in all patients with CSA (Fig. 1). Furthermore, postoperative needle EMG revealed that a significantly fewer

Table 1
Measurements of MUNIX and clinical measures in the patients with proximal-type CSA.

	Proximal-type CSA patients				Controls
Number of cases	22				41
Age range (years)	57.3 ± 6.6				49.3 ± 9.3
Height range (cm)	172.0 ± 4.4				169.9 ± 5.9
Duration (months)	22.9 ± 19.9				
	Data		Number of abnormalities		
	S side	Less-S side	S side	Less-S side	/
Abductor digiti minimi					
CMAP	9.5 ± 1.6	9.3 ± 1.7	/	/	9.7 ± 2.0
MUNIX	162.9 ± 46.6	164.7 ± 42.0	/	/	166.3 ± 40.2
MUSIX	62.1 ± 17.5	58.0 ± 8.6	5/22	/	59.2 ± 8.7
MRC	5.0 ± 0.0	5.0 ± 0.0	/	/	5.0 ± 0.0
Abductor pollicis brevis					
CMAP	9.6 ± 2.4	9.6 ± 2.5	/	/	9.8 ± 2.2
MUNIX	168.2 ± 45.6	169.5 ± 42.7	/	/	175.0 ± 45.7
MUSIX	58.6 ± 10.9	56.7 ± 6.8	2/22	/	57.5 ± 9.6
MRC	5.0 ± 0.0	5.0 ± 0.0	/	/	5.0 ± 0.0
Middle deltoid					
CMAP	4.7 ± 3.0	12.9 ± 3.5	13/21	/	13.1 ± 3.2
MUNIX	85.1 ± 42.0	250.7 ± 73.2	18/18	3/22	266.3 ± 67.8
MUSIX	69.8 ± 21.3	53.5 ± 11.5	5/18	2/22	50.1 ± 8.6
MRC	2.8 ± 1.5	5.0 ± 0.0	18/22	/	5.0 ± 0.0
Biceps brachii					
CMAP	4.8 ± 3.2	9.1 ± 3.3	9/22	/	10.2 ± 2.7
MUNIX	88.3 ± 66.3	169.0 ± 43.0	13/18	1/22	189.9 ± 56.0
MUSIX	76.7 ± 19.7	54.1 ± 12.5	9/18	1/22	55.2 ± 10.3
MRC	3.1 ± 1.5	5.0 ± 0.0	16/22	/	5.0 ± 0.0
Sum-score					
CMAP	69.6 ± 10.2		/		85.5 ± 17.2
MUNIX	1226.9 ± 205.3		/		1594.8 ± 314.4
MRC	36.0 ± 2.6		/		40.0 ± 0.0
HGS	40.7 ± 2.9	40.7 ± 2.8	/	/	42.5 ± 5.4
DASH	21.8 ± 9.4		/		/

MUNIX: Motor unit number index; **CSA**: Cervical spondylotic amyotrophy; **CMAP**: Compound muscle action potential; **MUSIX**: Motor unit size index; **MRC**: Medical research council score; **HGS**: Handgrip strength examination; **DASH**: Disabilities of the arm, shoulder and hand; **S side**: Symptomatic side; **Less-S side**: Less-symptomatic side. **a/b**: Where a is the number of patients from which abnormal measurements were recorded and b is the number of patients from which CMAP or MUNIX could be measured.

number of subjects in both CSA patient groups presented with spontaneous activities compared with preoperative needle EMG findings ($P < 0.05$, Tables 3 and 4). Both MUNIX measurements and clinical functional measures of patients with CSA between two assessments are listed in Tables 3 and 4, as well as in Supplementary Tables 5 and 6.

3.2.1. The patients with proximal-type CSA

MUNIX values increased significantly in both the Del and BB on the symptomatic side at 18.1 ± 3.1 (13–22) months after surgery (Table 3, Fig. 2), while the CMAP amplitude of both the Del and BB on the symptomatic side showed a marked increase between two assessments ($P < 0.05$) (Table 3).

MRC scores in both the Del and BB on the symptomatic side were significantly higher compared to the initial assessment, while DASH decreased obviously ($P < 0.05$) (Table 3). Fourteen (14/19, 73.7%) patients with proximal-type CSA presented with reduced DASH at the postoperative assessment, and 13 of these 14 patients showed recovery of MRC scores in at least one tested muscle. In contrast, five (5/19, 26.3%) patients failed to show any changes in clinical function between the two assessments. However, all MUNIX measurements (CMAP: 4.5 ± 2.7 vs. 6.2 ± 2.3, MUNIX: 60.8 ± 46.4 vs. 70.4 ± 44.0, MUSIX: 85.9 ± 21.8 vs. 104.4 ± 33.2) for the Del increased significantly on the symptomatic side of these five patients at the postoperative assessment ($P < 0.05$), while a significant increase in both MUSIX (67.0 ± 13.7 vs. 82.6 ± 24.8, $P < 0.05$) and CMAP (5.2 ± 3.1 vs. 6.2 ± 2.7, $P < 0.05$) for the BB on the same side without reduced MUNIX values (88.2 ± 66.3 vs. 90.2 ± 63.4, $P > 0.05$). Furthermore, four of these 5 patients presented with absent spontaneous activities in all tested muscles at the postoperative assessment.

3.2.2. The patients with distal-type CSA

Both MUSIX values and CMAP amplitudes for the APB and ADM, as well as MUNIX values for the ADM, increased significantly on the symptomatic side at 18.5 ± 4.0 (12–24) months after surgery ($P < 0.05$, Table 4; Fig. 2 and Supplementary Fig. 1), and these patients also showed a marked increase in both MUNIX values and CMAP amplitudes for symptomatic-side BB after surgery ($P < 0.05$, Supplementary Table 6; Fig. 2).

MRC scores for the symptomatic-side ADM were significantly higher than those at the initial assessment ($P < 0.05$, Table 4). Compared to patients with proximal-type CSA, fewer patients with distal-type CSA presented with improved clinical function at the postoperative assessment (7/18 vs. 14/19, $P < 0.05$). Nine (9/18, 50%) patients with distal-type CSA failed to show any changes in either clinical functional measures or MUNIX tests between the preoperative and postoperative assessments, and 2 of these 9 patients still presented with spontaneous activities in the APB and/or ADM on the symptomatic side after surgery. In addition, the other two (2/18, 11.1%) patients showed increased DASH scores, along with reduced MUNIX values consistent with this advancement in motor dysfunction (MUNIX sum-score: patient #1: 840 vs. 782; patient #2: 1428 vs. 1287), and both patients showed spontaneous activities in both the APB and ADM on the bilateral side.

Table 2
Measurements of MUNIX and clinical measures in the patients with distal-type CSA.

	Distal-type CSA patients				Controls
Number of cases	25				41
Age range (years)	52.4 ± 7.6				49.3 ± 9.3
Height range (cm)	171.3 ± 4.3				169.9 ± 5.9
Duration (months)	28.7 ± 35.5				
	Data		Number of abnormalities		
	S side	Less-S side	S side	Less-S side	/
Abductor digiti minimi					
CMAP	5.1 ± 2.3	9.1 ± 2.3	14/25	2/25	9.7 ± 2.0
MUNIX	64.0 ± 36.2	143.6 ± 59.8	18/23	7/25	166.3 ± 40.2
MUSIX	94.7 ± 23.1	69.3 ± 17.7	19/23	13/25	59.2 ± 8.7
MRC	3.6 ± 1.2	4.8 ± 0.4	19/25	5/25	5.0 ± 0.0
Abductor pollicis brevis					
CMAP	6.4 ± 1.6	9.6 ± 2.5	6/25	1/25	9.8 ± 2.2
MUNIX	94.2 ± 44.3	159.9 ± 61.1	13/25	4/25	175.0 ± 45.7
MUSIX	76.9 ± 23.2	65.2 ± 18.0	15/25	8/25	57.5 ± 9.6
MRC	4.6 ± 0.7	4.9 ± 0.3	9/25	2/25	5.0 ± 0.0
Middle deltoid					
CMAP	12.3 ± 3.8	13.1 ± 3.5	/	/	13.1 ± 3.2
MUNIX	245.4 ± 95.4	250.7 ± 73.2	6/25	/	266.3 ± 67.8
MUSIX	53.3 ± 11.1	54.0 ± 11.0	2/25	/	50.1 ± 8.6
MRC	5.0 ± 0.0	5.0 ± 0.0	/	/	5.0 ± 0.0
Biceps brachii					
CMAP	9.1 ± 3.6	9.9 ± 2.9	/	/	10.2 ± 2.7
MUNIX	170.5 ± 88.2	181.7 ± 49.4	7/25	/	189.9 ± 56.0
MUSIX	59.1 ± 14.8	55.0 ± 8.7	5/25	/	55.2 ± 10.3
MRC	4.9 ± 0.3	5.0 ± 0.0	3/25	/	5.0 ± 0.0
Sum-score					
CMAP	74.5 ± 12.7		/		85.5 ± 17.2
MUNIX	1319.8 ± 310.2		/		1594.8 ± 314.4
MRC	37.8 ± 2.2		/		40.0 ± 0.0
HGS	29.4 ± 6.4	40.5 ± 7.4	14/25	5/25	42.5 ± 5.4
DASH	10.3 ± 9.8		/		/

MUNIX: Motor unit number index; **CSA**: Cervical spondylotic amyotrophy; **CMAP**: Compound muscle action potential; **MUSIX**: Motor unit size index; **MRC**: Medical research council score; **HGS**: Handgrip strength examination; **DASH**: Disabilities of the arm, shoulder and hand; **S side**: Symptomatic side; **Less-S side**: Less-symptomatic side.
a/b: Where a is the number of patients from which abnormal measurements were recorded and b is the number of patients from which CMAP or MUNIX could be measured.

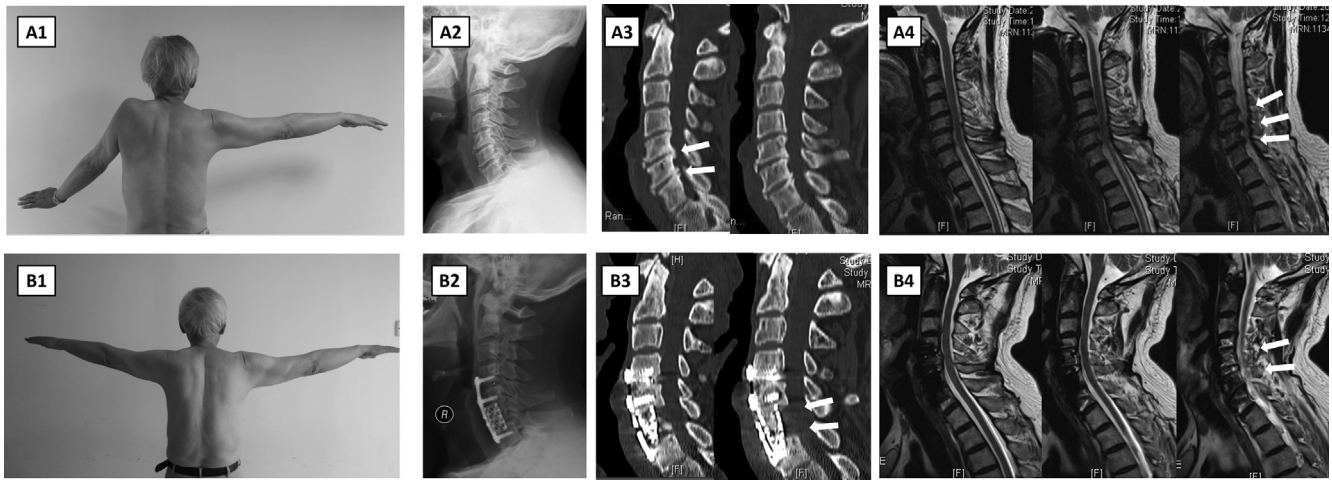

Fig. 1. A patient with proximal-type cervical spondylotic amyotrophy showed changes in both clinical motor function and imaging findings between before (A) and after the operation (B). Motor function improved significantly between these two assessments in this patient (A1 vs. B1), and postoperative imaging evaluation demonstrated that nerve compression by bony spur (A3-4 vs. B3-4), as well as cervical kyphosis (A4 vs. B4), were corrected at the surgical segments.

4. Discussion

The results of this study showed a clear decrement in MUNIX values in patients with CSA and significant correlation between reduced MUNIX values and motor impairment in these patients. Furthermore, we confirmed a marked increase in MUNIX values in patients with CSA within an 18-month period after operation. All of these results suggest the feasibility of MUNIX in quantitatively evaluating motor dysfunction, disease progression and treatment outcome in patients with CSA.

The MUNIX method used in this study is less time-consuming and well-tolerated, and it usually takes approximately thirty min-

Table 3
Follow-up measurements of both proximal muscles and clinical function in proximal-type CSA.

Number of patients	19					
Age range (years)	57.5 ± 5.7					
Height range (cm)	172.4 ± 4.6					
Duration (months)	19.5 ± 12.7					
	Initial test		Follow-up test		*P*-values*	
	S-side	Less-side	S-side	Less-S side	S-side	Less-S side
MUNIX test						
CMAP-Del	4.9 ± 2.9	13.5 ± 3.3	7.0 ± 3.2	13.8 ± 3.3	<0.01	0.24
MUNIX-Del	82.4 ± 41.5#	260.8 ± 67.4	101.7 ± 58.2#	264.4 ± 66.2	<0.01	0.66
MUSIX-Del	71.1 ± 21.1#	53.2 ± 10.5	81.2 ± 28.7#	53.0 ± 8.2	0.07	0.84
CMAP-BB	5.1 ± 3.0	9.6 ± 3.3	6.5 ± 2.8	9.7 ± 2.9	<0.01	0.84
MUNIX-BB	85.8 ± 67.5#	175.6 ± 39.4	92.8 ± 62.0#	183.7 ± 42.7	<0.01	0.24
MUSIX-BB	77.8 ± 20.3#	54.2 ± 11.1	82.2 ± 22.5#	52.5 ± 9.1	0.82	0.18
CMAP sum score	71.6 ± 9.0		75.4 ± 7.9		<0.01	
MUNIX sum score	1262.4 ± 186.3		1318.4 ± 182.6		<0.01	
MRC score						
Del	3.1 ± 1.4	5.0 ± 0.0	3.7 ± 1.0	5.0 ± 0.0	<0.01	/
BB	3.3 ± 1.3	5.0 ± 0.0	4.1 ± 1.2	5.0 ± 0.0	<0.01	/
MRC sum score	36.3 ± 2.3		37.8 ± 2.0		<0.01	
HGS examination						
HGS (kg)	40.6 ± 3.1	40.7 ± 2.9	40.6 ± 2.7	40.8 ± 2.9	0.80	0.48
DASH measure						
DASH	21.7 ± 9.8		9.7 ± 10.5		<0.01	
Needle EMG detection						
Spontaneous activities	19/19		2/19		<0.01	
Changes in MUAPs	19/19		19/19		/	

MUNIX: Motor unit number index; **CSA**: Cervical spondylotic amyotrophy; **CMAP**: Compound muscle action potential; **MUSIX**: Motor unit size index; **MRC**: Medical research council score; **HGS**: Handgrip strength examination; **DASH**: Disabilities of the arm, shoulder and hand; **Del**: Middle deltoid; **BB**: Biceps brachii; **S side**: Symptomatic side; **Less-S side**: Less-symptomatic side; **MUAP**: Motor unit action potential.

* *P*-values between the initial and follow-up tests.

\# Two patients failed to show MUNIX measurements in both Del and BB at the initial assessment, and these parameters could be measured in both of these two patients at the follow-up assessment.

Table 4
Follow-up measurements of both distal tested muscles and clinical function in distal-type CSA.

Number of patients	18					
Age range (years)	54.0 ± 6.9					
Height range (cm)	169.9 ± 4.1					
Duration (months)	29.5 ± 39.9					
	Initial test		Follow-up test		*P*-values*	
	S-side	Less-side	S-side	Less-side	S-side	Less-S side
MUNIX test						
CMAP-ADM	4.9 ± 2.5	9.0 ± 2.4	5.7 ± 2.3	9.1 ± 1.9	< 0.01	0.87
MUNIX-ADM	62.1 ± 33.9#	136.9 ± 60.9	66.7 ± 30.8#	138.6 ± 54.9	0.02	0.74
MUSIX-ADM	96.7 ± 22.9#	72.5 ± 17.8	101.6 ± 26.1#	71.5 ± 17.8	0.03	0.63
CMAP-APB	6.2 ± 1.6	9.5 ± 2.5	7.0 ± 1.6	9.7 ± 2.6	< 0.01	0.13
MUNIX-APB	91.6 ± 45.3	160.3 ± 66.9	96.4 ± 43.6	163.8 ± 69.5	0.06	0.44
MUSIX-APB	75.7 ± 19.9	65.2 ± 19.3	80.1 ± 20.0	65.4 ± 19.9	0.03	0.93
CMAP sum score	73.5 ± 14.3		77.1 ± 14.0		< 0.01	
MUNIX sum score	1292.5 ± 350.7		1331.2 ± 337.8		0.04	
MRC score						
ADM	3.5 ± 1.3	4.9 ± 0.3	3.8 ± 1.2	4.9 ± 0.3	0.01	/
APB	4.5 ± 0.7	4.7 ± 0.5	4.7 ± 0.6	4.7 ± 0.5	0.08	/
MRC sum score	37.4 ± 2.5		38.1 ± 2.1		0.01	
HGS examination						
HGS (kg)	28.8 ± 6.1	40.6 ± 8.2	29.1 ± 6.4	40.9 ± 7.7	0.41	0.48
DASH measure						
DASH	12.4 ± 10.8		10.1 ± 8.2		0.07	
Needle EMG detection						
Spontaneous activities	18/18		4/18		< 0.01	
Changes in MUAPs	18/18		18/18		/	

MUNIX: Motor unit number index; **CSA**: Cervical spondylotic amyotrophy; **CMAP**: Compound muscle action potential; **MUSIX**: Motor unit size index; **MRC**: Medical research council score; **HGS**: Handgrip strength examination; **DASH**: Disabilities of the arm, shoulder and hand; **ADM**: Abductor digiti minimi; **APB**: Abductor pollicis brevis; **S side**: Symptomatic side; **Less-S side**: Less-symptomatic side; **MUAP**: Motor unit action potential.

* *P*-values between the initial and follow-up tests.

\# Two patients failed to show MUNIX measurements in the symptomatic-side ADM at the initial assessment, and these parameters could be measured in one of these two patients at the follow-up assessment.

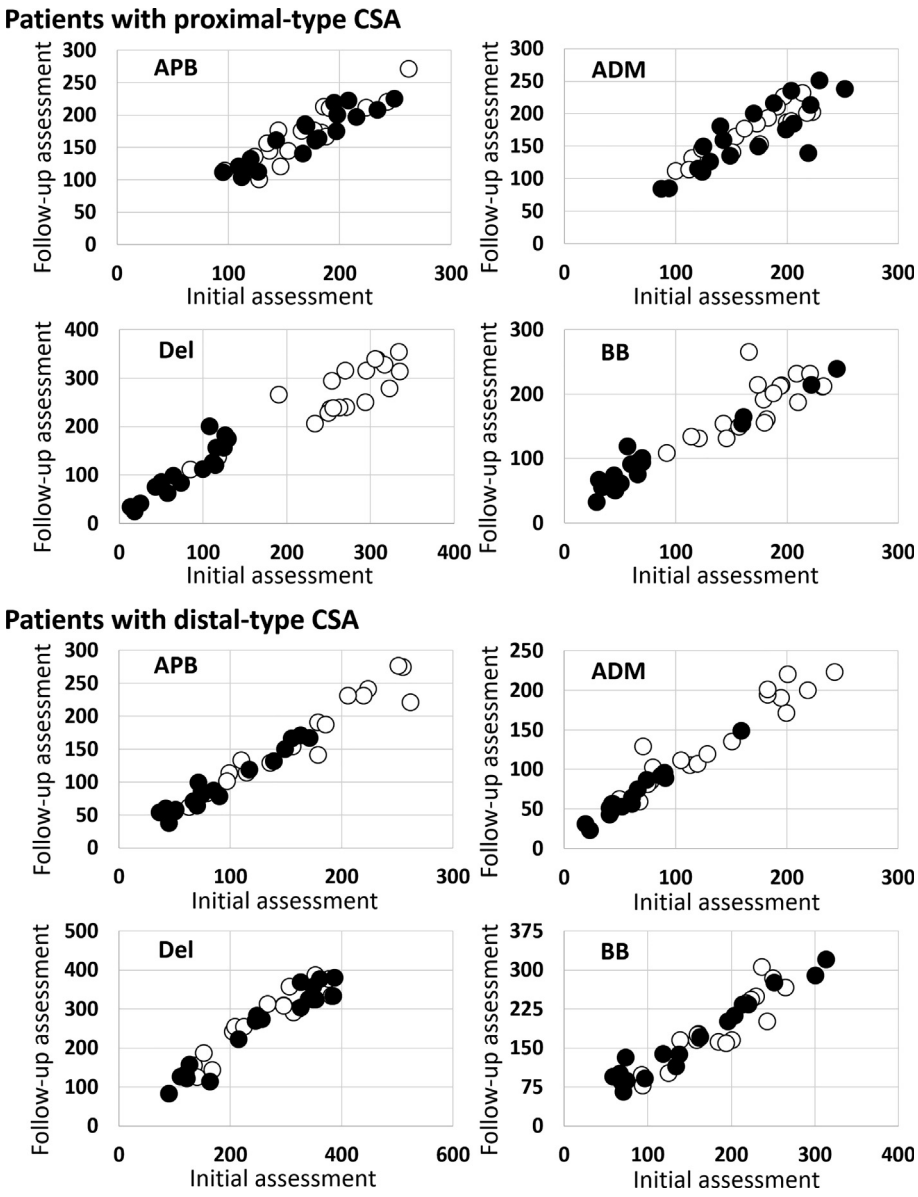

Fig. 2. Individual patients with CSA showed changes in MUNIX values (●: Symptomatic side; ○: Less-symptomatic side) between the initial assessments and 18-month follow-up assessments. Between these two assessments, MUNIX values increased significantly in both the Del and BB on the symptomatic side in patients with proximal-type CSA, and MUNIX values increased significantly in both the ADM and BB on the symptomatic side in patients with distal-type CSA. CSA: cervical spondylotic amyotrophy; MUNIX: motor unit number index; ADM: abductor digiti minimi; APB: abductor pollicis brevis; Del: middle deltoid; BB: biceps brachii.

utes to measure eight muscles in one subject. However, in order to make this method to be an available measure in CSA clinical trials, it is important that the results it produces are reliable. According to the results of both ICC and CCA, we identified the excellent levels of reproducibility of MUNIX measurements in both the control and CSA patient groups. Although a relatively higher COV of MUNIX values was found in the severely affected muscles in patients with CSA, which may be ascribed to low MUNIX values in these muscles, most tested muscles presented with COV values lower than 20%. Therefore, the findings of the present study are technically sound and reflect reliably functional motor units of the tested muscles.

In this study, the decrease in MUNIX values in CSA individuals was proportionally greater than that in CMAP amplitude for the same muscle, and some tested muscles presented with MUNIX abnormalities accompanied by normal CMAP amplitudes. These conditions were in agreement with those observed in the CMT, SMA and ALS (Escorcio-Bezerra et al., 2016; Querin et al., 2018; Bas et al., 2018), and the collateral innervation from remaining motor units may be main reason. A considerable increase in MUSIX values in CSA patients in this study further supported the existence of collateral re-innervation. Therefore, MUNIX may be more sensitive as an early index in detecting the loss of motor neurons than CMAP amplitude in patients with CSA, although the latter is much easier to obtain in daily clinical practice.

Although the motor dysfunction in CSA related to the loss of motor neurons was demonstrated to be well reflected by the estimation of MUNIX, approximately one-half of the tested muscles with clearly abnormal MUNIX measurements showed normal muscle strength in this study, indicating the motor neurons loss may appear before motor dysfunction occurs in CSA. Full functional compensation of the re-innervation may be one of the main reasons for this condition, and a significantly abnormal increase in motor unit size in all of these tested muscles with normal MRC scores provided more evidence of this compensatory process. According to previous studies, muscle strength can usually be kept in the normal range until decompensation of innervation, which

cannot occur until disintegration of LMNs reach 50–70% (Fukada et al., 2016; Zheng et al., 2017b). Therefore, compared with motor functional measures and CMAP amplitude, the estimation of MUNIX may provide a better clinical biomarker for evaluating the loss of motor neurons in CSA, even during early asymptomatic stage of CSA.

According to the Supplementary Table 4, there is an obvious negative correlation between MUNIX sum-scores and disease duration in both CSA patient groups, suggesting that the loss of motor neurons in CSA worsens as the disease advances. However, contrary to this result, we demonstrated significantly increased MUNIX values in most patients with distal- and proximal-type CSA within an 18-month period after operation. This finding suggests that ACDF procedures may be generally effective in the treatment of patients with CSA, consistent with previous studies (Fujiwara et al., 2006; Uchida et al., 2009; Inui et al., 2011; Wang et al., 2014). Equally important, although both HGS and DASH were similar between two examinations in approximately one-fourth of the patients with proximal-type CSA, the measurements of MUNIX tests in the mainly involved muscles of these patients showed significant improvements at the follow-up assessments. This condition may be ascribed to the clinical assessment are suboptimal to detect subtle response to the treatment (Philibert et al., 2017), and postoperative insufficient recovery time, especially in patients with distal-type CSA, may be another possible reason. Overall, although functional improvement is very important to the CSA patients in clinical practice, mildly improved MUNIX may provide additional unique information for monitoring treatment outcome and selecting treatment modalities.

In the present study, surgical prognosis in patients with proximal-type CSA was relatively superior to that in cases with distal-type CSA, and similar result was reported in previous studies (Uchida et al., 2009; Wang et al., 2014). Compared with noticeably increased MUSIX values in the severely affected muscles in distal-type CSA, the patients with proximal-type CSA mainly presented with a significant increase in MUNIX values without modification of motor unit size after surgical decompression, which may be attributed to the functional recovery of some motoneurons and/or ventral nerve root (Fatehi et al., 2017). Thus, the possibility for a relatively better surgical outcome in proximal-type CSA than that in distal-type CSA is that selective compression in proximal-type CSA may mainly cause the dysfunction of ventral nerve root and/or motor neurons rather than a loss of motor neurons. Therefore, the MUNIX technique may provide more information to help explaining the physiopathology of electrophysiological modifications than CMAP amplitude alone.

The results of the current study should be explained with caution because not all CSA patients can get a good surgical outcome. Furthermore, although MUNIX may a reasonable tool to assess and understand changes of motor function due to CSA and its subsequent surgery, many questions are left open, and more marked results might be obtained in future study with an increased number of patients with long-time follow-up and the combination of MUNIX and quantitative needle EMG. Another potential limitation of this study is that MUNIX measurements are easily influenced by both examiner and methods (Neuwirth et al., 2017; de Carvalho et al., 2018). Therefore, a standard protocol for positioning the electrodes and a fixed examiner were used in this study. Additionally, standardized instruction and a brief demonstration for isometric contraction were given to all subjects.

5. Conclusion

The MUNIX technique may provide a simple, well-tolerated and non-invasive additional test, that is complementary to conventional electromyography, for quantitatively evaluating the motor dysfunction related to motor neuron loss in CSA and for monitoring treatment outcome and disease advancement of CSA, even in the subclinical stage of this disease.

Declaration of Competing Interest

The authors report no conflicts of interest. The authors are solely responsible for the content and writing of this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2019.05.023.

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