

COMPOUND MUSCLE ACTION POTENTIAL AND MOTOR FUNCTION IN CHILDREN WITH SPINAL MUSCULAR ATROPHY

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ABSTRACT: Reliable outcome measures that reflect the underlying disease process and correlate with motor function in children with SMA are needed for clinical trials. Maximum ulnar compound muscle action potential (CMAP) data were collected at two visits over a 4–6-week period in children with SMA types II and III, 2–17 years of age, at four academic centers. Primary functional outcome measures included the Modified Hammett Functional Motor Scale (MHFMS) and MHFMS-Extend. CMAP negative peak amplitude and area showed excellent discrimination between the ambulatory and non-ambulatory SMA cohorts (ROC = 0.88). CMAP had excellent test–retest reliability (ICC = 0.96–0.97, $n = 64$) and moderate to strong correlation with the MHFMS and MHFMS-Extend ($r = 0.61–0.73$, $n = 68$, $P < 0.001$). Maximum ulnar CMAP amplitude and area is a feasible, valid, and reliable outcome measure for use in pediatric multicenter clinical trials in SMA. CMAP correlates well with motor function and has potential value as a relevant surrogate for disease status.

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Spinal muscular atrophy (SMA) is the most common inherited motor neuron disease. It is a progressive neuromuscular disorder that occurs with an incidence of 1 in 10,000 live births.^{1,2} Most patients with SMA have markedly reduced muscle strength and motor function across a breadth of severity ranging from profound generalized weakness to modest proximal muscle weakness. The spectrum of SMA is divided into groups based upon maximum achieved gross motor function: SMA type I defines those children who are unable to sit; type II defines those who achieve the ability to sit independently; and type III defines those

who, at least for a time, achieve the ability to walk.^{3–7} Recent advances in our understanding of mechanisms of disease pathogenesis in SMA research are driving the development of targeted therapeutic strategies. This in turn is increasing the need to develop and validate meaningful outcome measures and their surrogates for use in clinical trials.

Identification of reliable, objective outcome measures that closely reflect the underlying disease process, correlate with motor function, and are feasible for use in clinical trials in both children and adults with SMA are important in assessing the potential benefit of a therapy and in understanding its mechanism of action. Muscle weakness in SMA is clearly related to degree of innervation. However, compensatory mechanisms that result in changes in motor unit size, muscle fiber size, and recruitment may differ between individual patients and over the range of SMA phenotype severity.

We have previously demonstrated that disease severity in SMA is correlated with the degree of innervation as determined by maximum ulnar compound muscle action potential (CMAP) amplitude and motor unit number estimation.⁸ Although SMA is traditionally considered a proximal muscle disease, all 89 subjects in our initial study, regardless of degree of weakness or type, had clearly evident denervation in a distal muscle, the abductor digiti minimi. Even in those with CMAP amplitude values that overlapped with normal controls, changes in motor unit size and morphology suggested collateral reinnervation in this nerve–muscle group.

Maximum ulnar CMAP amplitude demonstrated excellent test–retest reliability in this single-center SMA natural history study, and it correlated with general functional status using a basic ordinal

Abbreviations: CMAP, compound muscle action potential; ICC, intra-class correlation coefficient; MHFMS, Modified Hammett Functional Motor Scale; NP, negative peak; ROC, receiver-operator characteristic; SMA, spinal muscular atrophy; SMN, survival motor neuron

Key words: compound muscle action potential, motor function, outcome measures, pediatrics, spinal muscular atrophy

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scale to depict overall gross motor function at the time of CMAP testing independently from SMA type.⁸ In this study, we provide additional data in support of maximum ulnar CMAP amplitude and area as a candidate surrogate of disease status and motor function in SMA. Furthermore, we document the performance characteristics, feasibility, and rationale for use of this outcome measure in children with SMA types II and III in the pediatric multicenter clinical trials setting.

METHODS

Participants. All data were obtained as a result of participation in the multicenter SMA CARNI-VAL trial (ClinicalTrials.gov ID NCT00227266). The SMA CARNI-VAL trial was a phase II multicenter trial involving two subject cohorts that evaluated the safety, tolerability, and efficacy of combined regimen of oral valproic acid and carnitine in patients with SMA types II and III.⁹ All participants had documented homozygous deletion of survival motor neuron 1 (*SMN1*) to verify diagnosis of SMA. The “non-ambulatory cohort” participants were SMA “sitters,” 2–8 years of age, and included children with SMA types II and III after loss of ambulatory function. The “ambulatory cohort” participants were SMA “standers and walkers,” 3–17 years of age, and included children with SMA type III who could still walk. Participation was excluded for any subject taking any pharmacological agent known to modify the disease or modulate expression of *SMN2* for at least 3 months prior to enrollment.

A total of 94 participants were enrolled in the SMA CARNI-VAL trial: 61 in the non-ambulatory cohort (ages 1.8–8.7 years, mean 4.3 ± 2 years) and 33 in the ambulatory cohort (ages 2.8–16.3 years, mean 7.3 ± 3.7 years). Data on the first screening visit (S1) were initially obtained from patients at all six CARNI-VAL sites. Upon review of the submitted waveforms and before any analysis, two of these sites’ studies were found to have frequent technical problems that were outside the boundaries defined by the protocol. Individuals at these sites were not certified in electrophysiology, and had more limited pre-trial experience using the protocol in children. However, board-certified electrophysiologists with more experience performed studies at the remaining four sites. Analysis was done on patient data obtained from only the latter four sites. Therefore, for the current report, we performed a cross-sectional analysis of data obtained at two screening visits for children with SMA types II and III who were enrolled at four of six clinical trial sites in the SMA CARNI-VAL trial to assess the feasibility, reliability, and validity of

CMAP amplitude and area as a surrogate of SMA disease burden, and to explore associations between CMAP and motor function.

Ethics Statement. Written informed parental consent (for participants <18 years) and assent (for participants ≥ 7 years) were obtained from all participants. The study was approved by the institutional review board at each clinical trial site participating in the SMA CARNI-VAL trial and contributing data to this study (University of Utah, Wayne State University, Ohio State Biomedical, and Johns Hopkins University).

Procedure. All assessments took place during the CARNI-VAL trial screening visits. Participants were assessed twice during a 4–6-week baseline screening period by the study’s principal investigator (for CMAP) and clinical evaluator (for motor function) at the study site where they were enrolled. Content, administration, and scoring directions for functional tests (MHFMS and MHFMS-Extend) and CMAP are available in detail at <http://smaoutcomes.org>.

CMAP. Maximum ulnar CMAP amplitude and area were obtained by recording from the abductor digiti minimi muscle following ulnar nerve stimulation at the wrist. All electrophysiological testing was performed by electromyographers who were experienced in the assessment of pediatric patients and who were blinded to previous test results. Maximum values for both negative peak (NP) amplitude and NP area were obtained from a total of five G1 electrode placements, using a disposable surface electrode with a 7×4 mm recording area (part no. 9013L0203 adhesive surface electrode; Alpine Biomed). Oral midazolam was used for anxiolysis at one center at the discretion of the site’s principal investigator and approved by local institutional protocol, for children in whom anxiety or discomfort was considered likely to interfere with reliable testing and to facilitate repeated evaluations. Dosing was 0.2–0.5 mg/kg, with a maximum of 5 mg per dose. At the other three centers, no medications were used. Children were instead distracted using standard techniques that were regularly in use at the sites for pediatric electromyography studies.

All original CMAP waveforms were printed and faxed to the data-coordinating center with the completed clinical research form for independent review. CMAP amplitude and area data from all sites were screened by a single reviewer to ensure strict adherence to protocol and ascertain accuracy of placement of markers for amplitude and area measurements. CMAP data were excluded for one or more of the following reasons: (1) less than three technically adequate waveforms associated with unique G1 electrode placement; (2) electrical

artifact precluding accurate amplitude and/or area measurements; and/or (3) initial positive deflection exceeding one third of the NP amplitude.

Motor Function. Motor performance was assessed using the Modified Hammersmith Functional Motor Scale (MHFMS) in the non-ambulatory cohort, and the MHFMS-Extend in the ambulatory cohort. The MHFMS is reliable and stable over at least a 6-month duration in non-ambulatory children with SMA types II and III.¹⁰ The MHFMS-Extend is comprised of the 20 original MHFMS gross motor items, plus an additional 8 higher-level gross motor items (ClinicalTrials.gov). Evaluators were trained to ensure they met specific criteria for standardization of performance and reliability for both the MHFMS and MHFMS-Extend.

The possible MHFMS score range was 0–40, and the MHFMS-Extend possible score range was 0–56. Specific test administration and scoring criteria for functional tests and CMAP amplitude and area are described in detail at www.smaoutcomes.org.

Statistical Analysis. Feasibility of CMAP amplitude and area was assessed by the percentage of participants who successfully completed the procedure at the four sites that provided CMAP data.

Validity was assessed by the ability of CMAP amplitude and area to discriminate between the non-ambulatory and the ambulatory cohorts using area under the receiver-operator characteristic (ROC) curve. The ROC curve provides a validity assessment, because it is a direct function of both sensitivity and specificity, where sensitivity is equivalent to assessing “convergent validity” and specificity is equivalent to assessing “divergent validity.”¹¹

Test–retest reliability of CMAP amplitude and area measurements from the first (S1) to the second (S2) screening visit was measured using the intraclass correlation coefficient (ICC).

To assess correlation between CMAP values (NP amplitude and area) and motor function scores (MHFMS and MFMS-Extend), we used Pearson’s correlation (r). Scores from the same visit were used to determine the relationship of CMAP amplitude and area to motor function; when available, data from the S2 visit was used in preference to that from S1.

RESULTS

Of the 69 participants at the four centers with CMAP amplitude and area data from the SMA CARNI-VAL trial, 68 participants had good quality CMAP data for at least one screening visit, including 44 from the non-ambulatory cohort and 24 from the ambulatory cohort (Table 1). Sixty-four participants had CMAP data obtained at both screening visits. Two patients had usable CMAP data from only one screening visit, and 3 patients

Table 1. Demographics of study participants with CMAP negative peak amplitude and area data.

Characteristic	Non-ambulatory cohort (N = 44)	Ambulatory cohort (N = 24)
Age, mean (years)	4.6	7.0
Age, SD (years)	2.2	3.3
Age, median (years)	4.0	7.2
Age, range (years)	1.8–8.6	2.8–15.3
Female [N (%)]	24 (55%)	14 (58%)
Non-Hispanic [N (%)]	39 (88%)	21 (87%)

CMAP, compound muscle action potential; SD, standard deviation.

had only one screening visit with CMAP data. CMAP NP amplitude, expressed as mean \pm SD (range), was 2.3 ± 1.7 (0.5–7.6) mV for the non-ambulatory cohort and 5.5 ± 2.6 (1.2–10.4) mV for the ambulatory cohort. CMAP NP area mean \pm SD (range) was 5.5 ± 4.6 (0.7–19.7) mVms for the non-ambulatory cohort and 14.9 ± 7.7 (2.6–38.3) mV for the ambulatory cohort. For functional motor testing, all 68 participants with CMAP amplitude and area data completed either the MHFMS [44 participants in the non-ambulatory cohort; MHFMS score mean \pm SD (range): 18.8 ± 9.6 (3–38)], or the MHFMS-Extend [24 participants in the ambulatory cohort; MHFMS-Extend score mean \pm SD (range): 47.3 ± 5.5 (36–56)].

Feasibility. CMAP data were successfully obtained from 99% (68 of 69) of participants during at least one screening visit during the CARNI-VAL trial at the four clinical trial sites that rigorously followed the CMAP amplitude and area study protocol. In addition, adequate CMAP amplitude and area values were obtained for 93% (64 of 69) of participants during both screening visits S1 and S2.

Validity. Validity was established with an ROC analysis between the CMAP and cohort membership (ambulatory vs. non-ambulatory). For CMAP NP amplitude, the ROC was 0.88, and for CMAP NP area the ROC was also 0.88. Using the Hosmer and Lemeshow¹² rule-of-thumb for interpreting ROC, a value between 0.8 and 0.9 is considered excellent discrimination; therefore, validity of CMAP NP amplitude and area as a measurement of innervation in SMA was clearly established. The discrimination, or separation, between the two cohorts is clearly illustrated in Figures 1 and 2.

Test–Retest Reliability. Test–retest reliability between the first and second screening visit CMAP NP amplitude was very high when all patients were analyzed as one group [ICC = 0.96, confidence interval (CI) = 0.93–0.97], as well as within the non-ambulatory cohort alone (ICC = 0.94, CI = 0.89–0.97) and the ambulatory cohort alone (ICC = 0.93, CI = 0.85–0.97). Similarly, test–retest

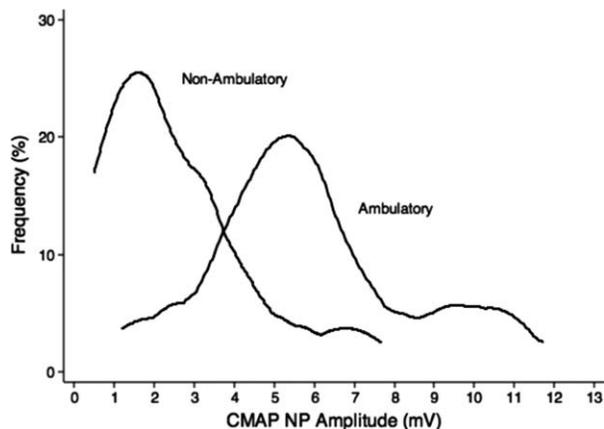


FIGURE 1. Smoothly drawn histograms for the two cohorts, also called kernel density plots, illustrating the separation of the two cohorts on the CMAP negative peak amplitude.

reliability between the first and second screening visit CMAP NP area was very high when all patients were analyzed together (ICC = 0.97, CI = 0.95–0.98), as well as within the non-ambulatory cohort alone (ICC = 0.96, CI = 0.93–0.98) and the ambulatory cohort alone (ICC = 0.94, CI = 0.88–0.98). The percent difference between first and second screen measurements had an absolute mean \pm standard deviation (SD) of 0.5 ± 0.6 mV for CMAP NP amplitude and 1.1 ± 1.4 mVms for CMAP NP area. These absolute differences correspond to percent differences of $16.0 \pm 17.4\%$ and $15.8 \pm 19.5\%$, respectively. Within the non-ambulatory cohort, the difference between first and second screen measurements for CMAP NP amplitude was 0.4 ± 0.4 mV ($19.2 \pm 19.0\%$), and for CMAP NP area it was 0.9 ± 0.9 mVms ($19.2 \pm 19.0\%$). Within the ambulatory cohort, the difference between first and second screen measurements for CMAP NP amplitude was 0.6 ± 0.7 mV ($10.2 \pm 12.3\%$), and for CMAP NP area it was 1.4 ± 1.9 mVms ($8.5 \pm 9.1\%$).

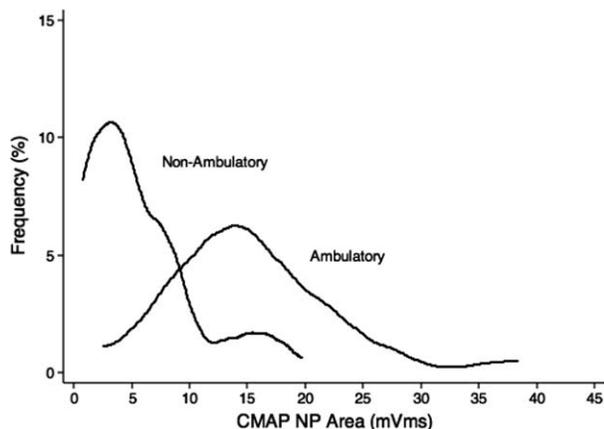


FIGURE 2. Smoothly drawn histograms for the two cohorts, also called kernel density plots, illustrating the separation of the two cohorts on the CMAP negative peak area.

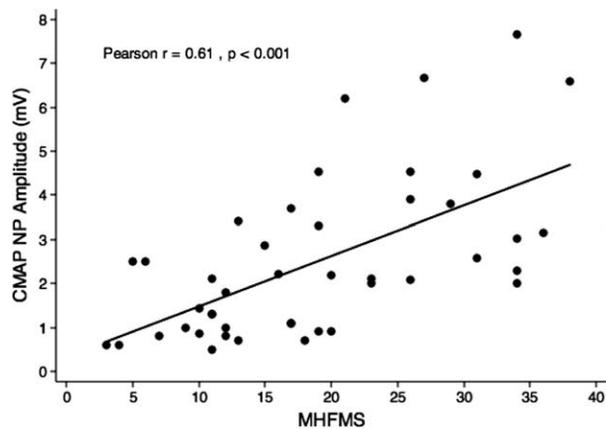


FIGURE 3. Scatterplot demonstrating correlation between maximum CMAP negative peak amplitude and MHFMS scores for the non-ambulatory cohort.

Correlation with Motor Function. CMAP NP amplitude and area values demonstrated a positive correlation with motor function in both cohorts ($r = 0.61$ – 0.73 , $P < 0.001$). When cohorts were evaluated separately, CMAP NP amplitude and area both correlated with the MHFMS motor function scores from the 44 participants in the non-ambulatory cohort (Figs. 3 and 4) and with the MHFMS-Extend scores from the 24 participants in the ambulatory cohort (Figs. 5 and 6).

DISCUSSION

Data from this multicenter trial in children with SMA types II and III show that maximum ulnar CMAP NP amplitude and area is: (1) a feasible, valid, and reliable physiological outcome measure; and (2) correlates with motor function. Maximum ulnar CMAP NP amplitude and area can thus be offered as valid and well-tolerated surrogate measures of innervation and hence disease burden in pediatric multicenter trial settings within the range of severity and age of subjects included in this trial.

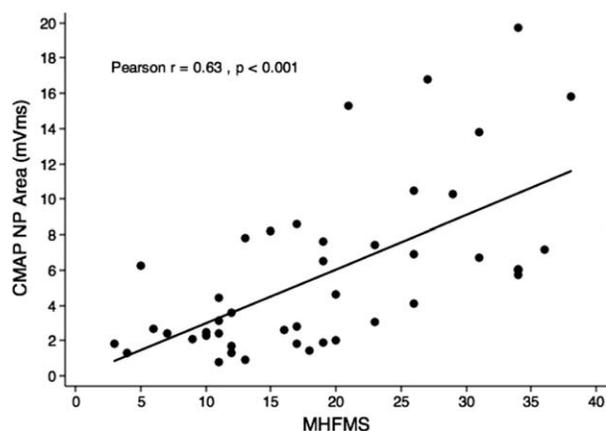


FIGURE 4. Scatterplot demonstrating correlation between maximum CMAP negative peak area and MHFMS scores for the non-ambulatory cohort.

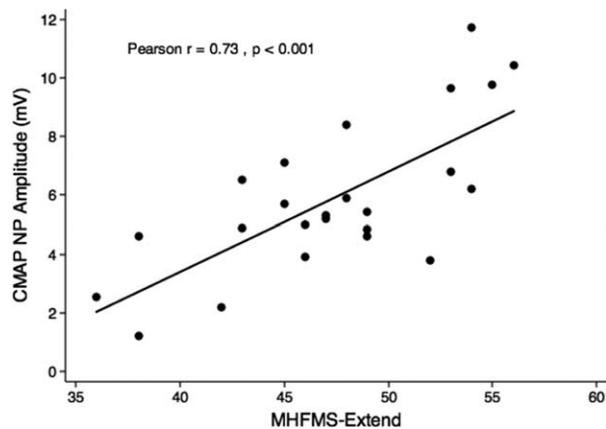


FIGURE 5. Scatterplot demonstrating correlation between maximum CMAP negative peak amplitude and MHFMS-Extend scores for the ambulatory cohort.

Although maximum CMAP typically refers to the maximum CMAP obtained from a single G1 electrode placement, actual values can vary by as much as 30% in this setting with electrode removal and replacement, reducing the reliability of the CMAP as an outcome measure. However, by specifying a minimum of at least three distinct G1 electrode placements, variability was further reduced by about half, to an average percent difference of 16%, such that its feasibility for use as an outcome measure to assess the combined nerve–muscle function was substantially improved. The three distinct G1 placements required by our protocol may have contributed in part to the high levels of test–retest reliability of CMAP NP amplitude and area observed in this study.

We initially chose the ulnar nerve–hypothenar muscle group for its ease of applicability across the entire range of disease severity and age from newborns to adult subjects with SMA.⁸ The current findings support the use of ulnar maximum CMAP NP amplitude and area in SMA. CMAP is noninvasive, easier to perform, requires minimal time and cooperation, is better tolerated by younger patients, and is less vulnerable to variation in operator expertise as compared with motor unit number estimation. Therefore, from a practical standpoint, it is preferable to motor unit number estimation in the pediatric clinical trials setting.

In an earlier investigation, we demonstrated a correlation with gross motor functional status using a simple ordinal scale in a single-site study.⁸ The present study extends that observed correlation and has shown that CMAP NP amplitude and area has a moderate to strong correlation with the SMA disease–specific graduated functional motor scales, the MHFMS and MHFMS-Extend. Higher CMAP amplitude and area values were associated with higher motor function even when each cohort

was analyzed individually, providing further evidence for CMAP NP amplitude and area as a valid measure of disease severity within study cohorts and for its power to discriminate more discrete levels of function given the smaller sample sizes and the constrained limits of function of each cohort.

Additional work remains to be done to fully assess the clinical utility of CMAP NP amplitude and area markers of disease over time and as predictors or measures of change. This measurement may also be of value to patients younger or older, or weaker or stronger, than the groups evaluated in this trial. CMAP NP amplitude and area as an outcome measure for clinical trials depends upon the hypothesized mechanism of therapeutic action. Therapies that would alter the number of functioning motor units, their capacity for collateral reinnervation, fidelity of junctional or sprout transmission, or muscle fiber bulk could potentially alter CMAP NP amplitude, whereas other potential targets of therapy may not.¹³

At present, with no established specific treatment for SMA, it is not possible to evaluate the extent to which CMAP NP amplitude and area is responsive to the change associated with an effective therapy. Nonetheless, CMAP assessment has potential value for clinical trial design in several ways. A change in CMAP NP amplitude or area might provide an early “read out” or “biomarker” of efficacy that could prove a robust surrogate for disease status in younger children who are not able to cooperate reliably with pulmonary function testing, myometry, or certain tests of motor function, such as the 6-minute walk test. CMAP NP amplitude and area also might prove useful as a means to stratify patient groups, or define potentially responsive patients within a window of values that restrict patients with expected ceiling or floor constraints for the primary outcome measure

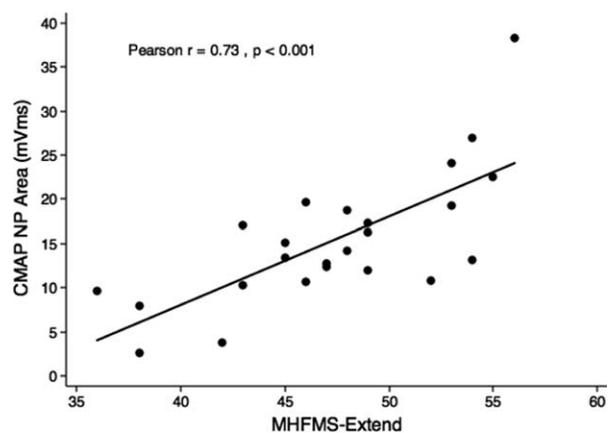


FIGURE 6. Scatterplot demonstrating correlation between maximum CMAP negative peak area and MHFMS-Extend scores for the ambulatory cohort.

chosen for a given trial. Given the feasibility, validity, reliability, and correlation with motor function, CMAP NP amplitude and area is a strong candidate for use as a relevant surrogate for disease status and motor function in future clinical trials.

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