

Research article

# Time-Efficacy in SMA Type 1 and 2 Cases Treated with Nusinersen

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**Abstract:** Spinal muscular atrophy is a neuromuscular degenerative disorder characterized by progressive apoptosis of motor neurons, with severe weakness and bulbar dysfunction. The aim of the study was to analyze the correlations between the moment of initiation of treatment (nusinersen) and clinical evolution, and also the change of electrophysiological parameters and motor scales, followed up for 2 years. This study was carried out between 2018 and 2022 on 60 SMA children (29 girls, 31 boys), (29 type 1 and 31 type 2; 29 with 2 copies of SMN2, 29 with 3 copies, and 2 with 4 copies), aged between 3 weeks and 196 months, divided into 2 groups according type of SMA. For both types of SMA, statistically significant negative correlations were found between the elapsed interval from the onset of the disease to the initiation of treatment and upper motor acquisitions (type 1:  $p < 0.0001$ ,  $r = -0.713$ , type 2:  $p < 0.001$ ,  $r = -0.560$ ) and between age at the beginning of treatment and improvement in motor function (type 1:  $p < 0.0001$ ,  $r = -0.726$ , type 2:  $p < 0.001$ ,  $r = -0.553$ ). For patients with type 2 SMA, a negative correlation was also identified between age at the time of onset and motor evolution ( $p < 0.05$ ,  $r = -0.378$ ). Electrophysiological parameters were strongly positive correlated with motor improvement ( $p < 0.0001$ ,  $r = 0.600$ ). Our study established the necessity of early SMA diagnosis and therapy beginning, and demonstrated that Compound Motor Action Potential can be a predictive factor in the disease's progression.

**Keywords:** Compound Motor Action Potential; motor scales; Spinal Muscular Atrophy

## 1. Introduction

Spinal muscular atrophy (SMA) is a neuromuscular degenerative disorder with autosomal recessive genetic transmission (most commonly homozygous deletion of exons 7 and 8 of the SMN1 gene) characterized by reduced functional SMN protein, progressive apoptosis of spinal and brainstem motoneurons, causing weakness and bulbar dysfunction [1–3].

More than 95% of SMA cases are due to the biallelic mutation of the SMN1 gene [4] on chromosome 5q (homozygous deletion) or the heterozygous compound or punctiform mutations [5]. The SMN2 gene [6] located on chromosome 5 contributes to a lesser extent

to the production of SMN protein and implicitly contributes to the phenotype of the disease [7].

The incidence of the disease is 1:6000-11000 newborns, making it one of the most common genetic causes of infant mortality [8]. Despite the rarity of the disorder, SMA has a significant impact on the affected individuals and their families. Understanding the causes, pathophysiology, clinical presentation, and management of SMA is essential for improving the diagnosis and treatment of this debilitating disorder.

The frequency of healthy carriers in the general population is 1:51 [9].

SMA is classified into five types, based on the age of onset and the severity of symptoms.

- Type 0 [10,11], the most severe form of SMA, is the rarest form of 5q-SMA, accounting for less than one percent of all cases. The onset of the disease is prenatal, manifesting itself by absence of fetal movements. The infants are born with severe hypotonia and weakness, facial diplegia, contractures, absent deep tendon reflexes, dysphagia and respiratory failure. A significant proportion of these patients also exhibit congenital cardiac abnormalities. Death occurs in the earliest weeks of life, before to 6 months of age [11,12].

- Type 1 [13], also known as Werdnig-Hoffmann disease, is usually diagnosed in infants. SMA type 1 patients develop symptoms around 0–6 months of age [14], truncal weakness, proximal limb weakness (lower>upper), bulbar dysfunction [15], respiratory failure [16], and poor feeding [17]. Patients with weak intercostal and chest wall muscle along with spared diaphragm strength have a bell-shaped chest deformity and paradoxical breathing [18]. There are fasciculations of the tongue, but no involvement of the face and ocular muscle [19]. Patients have normal cognitive function. They did not develop the capacity to sit independently (“non-sitters”) [20], and mortality frequently occurred before the age of 2 [21]. Most type 1 patients have between one and two copies of SMN2 gene. These newborns have a poor survival outlook [22].

- Type 2 [23], also known as intermediate SMA, is diagnosed in infants aged 6 to 18 months, and is characterized by the ability to sit but not walk [24]. Patients with SMA type 2 exhibit weakness and typically have three copies of SMN2 [25]. Patients typically acquire the capacity to sit unassisted (“sitters”), by the age of nine months, but may later lose this skill and be unable to stand or walk on their own. The clinical examination reveals predominately proximal weakness, which is more pronounced in the lower extremities, as well as fine distal tremor. In severe forms, respiratory insufficiency and dysphagia are frequently observed. Weak axial musculature may exacerbate restrictive lung illness and respiratory insufficiency [12] by contributing to the development of severe scoliosis.

- Type 3 [26], or Kugelberg-Welander disease is diagnosed in children between 2 and 17 years of age, and it is characterized by the ability to walk but with difficulty [27].

- Type 4, or adult-onset SMA, is the mildest form [28] and is diagnosed in individuals over 18 years of age and is usually characterized by mild symptoms such as muscle weakness [29].

Face and oculomotricity are not affected [30,31].

The diagnosis of spinal muscular atrophy (SMA) is based on a combination of clinical, laboratory and genetic findings.

The clinical criteria for SMA include the presence of muscle weakness and atrophy, as well as the age of onset and the pattern of muscle involvement.

The laboratory criteria for SMA include: electromyography (EMG) which can show signs of denervation (loss of nerve input) in affected muscles [32]; nerve conduction studies (low amplitudes on motor nerves), a nerve biopsy, which can show signs of degeneration in affected nerves [33].

Genetic testing is an essential tool in the diagnosis and management of spinal muscular atrophy (SMA). The genetic cause of SMA is a biallelic mutation or deletion in the survival motor neuron 1 (SMN1) gene located on chromosome 5q. There are two methods used in our center for genetic diagnosis:

- Multiplex ligation-dependent probe amplification (MLPA) is a modern quantitative molecular method. Applied in SMA cases, it improves diagnostics by simultaneously identifying the number of copies of several target sequences in the SMN1 gene and in nearby genes, SMN2 [34].

- qPCR uses the quantitative polymerase chain reaction to genotype the anterior spinal muscular atrophy (SMA) mutation in neonates on a dried blood sample. The test only identifies SMA homozygotes [35].

Genetic testing for SMA can be done on a variety of samples, including blood, saliva, and amniotic fluid, results being usually available within a few weeks. Genetic testing for SMA is not only for diagnosis, but also for carrier testing and prenatal diagnosis. Genetic counseling is recommended for individuals and families considering genetic testing for SMA, as the results of the test can have significant implications for the individual and the family.

The diagnosis of SMA can be challenging, and a multidisciplinary approach involving a neurologist, a geneticist, and a physical therapist is often required. Although the diagnosis of SMA is established by genetic testing, in medical practice, electrophysiological evaluation is a fast method, useful for differential diagnosis with the other causes of peripheral motor suffering, such as congenital peripheral neuropathies, myopathies or neuromuscular junction diseases [36].

Electrophysiological studies, such as electromyography (EMG) and nerve conduction studies (NCS), provide important information on the function of the motor neurons and the peripheral nerves in spinal muscular atrophy (SMA) [37,38]. EMG and NCS are not diagnostic tests, instead they are supportive tests that help to establish the diagnosis, and are helpful in differentiating SMA from other neuromuscular disorders. One of the key electrophysiological parameters that can be assessed in SMA is the distal amplitude of the compound muscle action potential (CMAP). CMAP is the summation of the action potentials coming from the motor fibers in a motor area, obtained by supramaximal stimulation of a peripheral motor nerve [39]. CMAP amplitude indicates the number of viable axons and implicitly the number of functional motoneurons [27,40], this being correlated with the clinical picture. In SMA, the distal amplitude of the CMAP is typically reduced, reflecting the loss of motor neurons and the subsequent decrease in the number of functioning motor units [41]. Rapid regression of motor acquisitions in type 1 of the disease was shown to be correlated with a marked decrease in the CMAP amplitude, being a predictive factor of unfavorable evolution. With stabilization of the disease in both type 1 and type 2, CMAP is maintained at steady low values. It was initially used as marker of the onset of the disease, later as marker for tracking the evolution in patients treated or untreated with innovative therapies (ASO and gene therapy) [42]. Reduced distal CMAP amplitudes, especially when accompanied by normal or slightly prolonged distal latencies, are considered a highly specific marker of axonal degeneration in SMA. This is particularly useful for differentiating SMA from other muscle disorders that can present with similar clinical symptoms, such as congenital myopathies or muscular dystrophies.

#### Current Treatments:

- Antisense oligonucleotide (ASO) therapy Nusinersen (Spinraza) is approved for the treatment of SMA types 1, 2, and 3. It is a SMN2 pre-mRNA targeted treatment for SMA designed to bind to the intronic splice silencer site in intron 7 of SMN-2 pre-messenger RNA, thereby boosting exon 7 inclusion at the SMN2 messenger RNA level [43–46]. It was approved on December 2016 for United States of America and May 2017 for Europe. Nusinersen increases the production of the survival motor neuron (SMN) protein, which is essential for the survival and function of motor neurons.

Nusinersen is administered directly into the spinal canal via a lumbar puncture.

The initiation starts with 4 administrations in 2 months (days 1-14-30-60), then 1 administration every 4 months. Post-administration adverse events related to nusinersen may occur after lumbar puncture (i.e., headache, vomiting, fever, low back pain, etc.) [47–51].

- In order to increase ASOs therapeutic efficiency, their delivery has to be closely monitored, as a matter of selectively targeting the tissue and adjusting the dose to the optimal values. By intravenous administration, the first barrier is represented by the vascular endothelium, the size of the particles limiting the process of simple diffusion. Another delivery strategy for ASOs is represented by nanoparticle systems using conjugated carriers enabling the selective delivery, including cell-penetrating peptides and tricyclic structures. Despite these strategies, the blood brain barrier remains the ultimate impenetrable territory, requesting extremely high and toxic systemic doses in order to reach adequate central concentrations. For this particular class of therapeutics, intrathecal or intraventricular administration is highly effective and safe, assuring a targeted delivery at the level of brain parenchyma and a uniform distribution throughout the central nervous system, the effective concentrations being rapidly reached. Nusinersen is administered intrathecally, with the first four loading doses being given in the first two months, then one injection every four months [47,52]. Nusinersen was found to be extremely efficient in improving motor function [48,49]. Several studies have shown that nusinersen-treated patients had variable respiratory [50] and nutritional status improvements.

- Typically, dose-dependent, the toxicity of ASOs may be hybridization-dependent either on-target, as a result of target RNA binding and modification, or off-target, as a result of ASO binding to an undesired target. Protein-ASO interactions connected to chemical changes of the ASO cause hybridization-independent toxicities, which include systemic side effects such as fever and arthralgias, thrombocytopenia, proximal renal tubular damage, and hepatotoxicity [47–51].

The management of SMA requires a multidisciplinary approach involving a neurologist, a geneticist, a physical therapist, a respiratory therapist, a nutritionist and an orthopedic surgeon.

The objective of this study was to analyze the correlations between the moment of initiation of the treatment and the clinical evolution, but also the change in the electrophysiological parameters in relation to the scores on the motor scales, followed up for 2 years.

## 2. Results

In the group of 29 patients diagnosed with SMA type 1, 25 patients had two copies of the SMN2 gene and 4 patients had three copies of the SMN2 gene. All patients were symptomatic and characterized as "non-sitters" at the time of the first examination. The electrophysiological study, CMAP, revealed low values of amplitude between 0.395 +/- 0.365 mV (T0) and 1.555 +/- 1.345 (T26). The score on the CHOP INTEND scale, which is a functional scale specific for SMA type 1, was 33 +/- 26 (T0) and 39 +/- 25 (T26). The initiation of treatment with nusinersen was variable and occurred between 1 and 25 months from the onset of symptoms.

The group of patients diagnosed with SMA type 2 was highly diverse and consisted of 31 subjects. All patients were characterized as "sitters" at the beginning of treatment. The group was composed of 25 patients with three copies of the SMN2 gene, 4 patients with two copies of the SMN2 gene, and 2 patients with four copies of the SMN2 gene. The CMAP values were between 2.44 +/- 2.35 mV (T0) and 27 +/- 25 mV (T26). The score on the HFMSE scale, which is a functional scale specific for SMA type 2, was between 18.5 +/- 17.5 (T0) and 27 +/- 25 (T26). The onset of symptoms occurred between 7-18 months, but the interval between the onset of symptoms and the start of therapy was extremely variable, ranging from 2 to 80 months. After two years of treatment with nusinersen, 72.4% of patients with SMA type 1 became "sitters" while 29.1% of patients with SMA type 2 became "walkers" (as shown in Figure 1).

Figure 1. Clinical evolution in SMA type 1 and 2 patients from initial examination (T0) to final examination (T26) with highlighting of clinical status (non-sitter, sitter, and walker)

At the initial examination (T0), of the 29 patients with 2 copies of the SMN2 gene, 86% were "non-sitters" and 14% were "sitters." At the final examination (T26), 21% remained "non-sitters", 65% became "sitters" from "non-sitters", 11% remained "sitters" and 3% became "walkers" from "sitters" (as shown in Figure 2).

Similarly, at T0, of the 29 patients with 3 copies of the SMN2 gene, 14% were "non-sitters" and 86% were "sitters." At T26, 7% remained "non-sitters", 7% became "sitters" from "non-sitters", 62% remained "sitters" and 24% became "walkers" from "sitters" (as shown in Figure 2).

Both patients with 4 copies of SMN2 were "sitters" at T0 and, at T26 one of them became "walker" while the other one remained "sitter" (as shown in Figure 2).

Figure 2. Clinical evolution in SMA type 1 and 2 patients, according to numbers of SMN2 copies, from initial examination (T0) to final examination (T26) with highlighting of clinical status (non-sitter, sitter, and walker).

The electrophysiological study revealed that the amplitudes of the CMAP increased slightly over time for patients with 2 copies of the SMN2 gene. However, the amplitudes remained low, around 1mV, at the final examination (T26), as shown in Figure 3.

Figure 3. Evolution of CMAP amplitudes in patients with 2 copies of SMN2, from initial examination (T0) to final examination (T26).

The mean of CMAP amplitudes in patients with 2 copies of SMN2 increased from 0.42 mV at the initial examination (T0) to 1.07 at the final examination (T26), while the minimum and maximum values increased from 0.03 mV at T0 to 0.21 mV at T26 and, from 2.8 mV at T0 to 3.7 mV at T26, respectively.

For patients with 3 copies of the SMN2 gene, the average amplitudes were higher and maintained an upward trend, reaching an average value of 2.9 mV at the final examination (T26), as seen in Figure 4.

Figure 4. Evolution of CMAP amplitudes in patients with 3 copies of SMN2, from initial examination (T0) to final examination (T26).

The mean of CMAP amplitudes in patients with 3 copies of SMN2 increased from 1.19 mV at the initial examination (T0) to 1.88 at the final examination (T26), while the minimum and maximum values increased from 0.14 mV at T0 to 0.22 mV at T26 and, from 4.8 mV at T0 to 5.1 mV at T26, respectively.

Both patients with 4 copies of the SMN2 gene had a moderate increased amplitude, with an average value of 0.61 mV, as shown in Figure 5.

Figure 5. Evolution of CMAP amplitudes in patients with 4 copies of SMN2 from initial examination (T0) to final examination (T26)

The mean of CMAP amplitudes in patients with 4 copies of SMN2 increased from 0.83 mV at the initial examination (T0) to 1.44 at the final examination (T26), while the minimum and maximum values increased from 0.8 mV at T0 to 1.34 mV at T26 and, from 0.85 mV at T0 to 1.53 mV at T26, respectively.

Analyzing the influence of the time elapsed between the onset of the disease and the initiation of treatment, it was shown that there is a significant correlation between this variable and the motor evolution of patients with SMA types 1 and 2. The negative correlation coefficient values of -0.713 and -0.560 for SMA type 1 and type 2, respectively, indicate that as the time elapsed between onset and treatment initiation increases, the motor function of patients with SMA types 1 and 2 worsens.

In the case of patients with SMA type 1, a statistically significant negative correlation was established between the disease's onset age and the patients' treatment-related progress ( $p < 0.05$  and  $r = -0.378$ ).

The patient's young age at the initiation of the treatment has a favorable influence on the evolution of the disease. Thus, we found a strong negative correlation between age at initiation of the treatment and the evolution on motor scales, for both types of SMA (type 1:  $p < 0.0001$  and  $r = -0.726$ ; type 2:  $p < 0.001$  and  $r = -0.553$ ).

The electrophysiological parameter CMAP at initial evaluation (T0) has found to be highly significant correlated with motor status evolution ( $p < 0.001$  and  $r = 0.600$ ).

### 3. Discussion

Our study followed the evolution of a group of patients diagnosed with SMA type 1 and 2, who were all symptomatic at the time of starting the treatment, specifically after the onset of motor regression. The results of our study highlighted the importance of the timing of introducing nusinersen treatment, as the evolution of the patients was found to depend on it.

We have shown that the shorter the time between the onset of symptoms and the initiation of treatment, the greater the recovery of motor regression and the gain of new motor acquisitions. This indicates that the earlier the treatment is started, the better the chances of preserving the remaining motor neurons and slowing down the progression of the disease. This highlights the importance of early diagnosis and prompt initiation of treatment for SMA patients in order to achieve the best possible outcome.

It is also important to note that the support of the rehabilitation and physical therapy along with the treatments plays a crucial role in the recovery and management of the SMA patients, it should be considered as a part of the overall treatment plan.

Thus, in our study, for SMA type 1 patients, a statistical analysis was performed on two parameters: the time difference between the initiation of treatment and the onset of the disease, and upper motor acquisitions - specifically, the transition from "non-sitters" to "sitters". The analysis revealed a strong negative correlation between these two parameters ( $p < 0.0001$ ,  $r = -0.713$ ). This means that the shorter the time between the onset of symptoms and the initiation of treatment, the greater the recovery of motor regression and the gain of new motor acquisitions.

Furthermore, the analysis showed that the younger the age at onset, the better the motor function was observed, with a statistically significant correlation between these two parameters ( $p < 0.005$ ,  $r = -0.378$ ). This suggests that the earlier the onset of symptoms, the better the chances of preserving the remaining motor neurons and slowing down the progression of the disease. Additionally, the study found that the younger the age at which the treatment was initiated, the better the motor development, with a strong negative correlation between these two parameters ( $p < 0.0001$ ,  $r = -0.726$ ).

In summary, our study found that for SMA type 1 patients, the earlier the treatment is started, the better the chances of preserving the remaining motor neurons, slowing down the progression of the disease and recovering motor regression, with a strong negative correlation between the time of initiation of treatment and the onset of the disease, and upper motor acquisitions, as well as a strong negative correlation between the age of onset and the age of initiation of treatment.

In the group of patients with SMA type 2, the statistical analysis of the same parameters showed that the evolution is all the better the shorter the interval between the onset of the disease and the initiation of treatment, the transition from "sitters" to "walkers" being statistically correlated with this ( $p < 0.001$ ,  $r = -0.560$ ).

This means that for SMA type 2 patients, similar to SMA type 1 patients, the shorter the time between the onset of symptoms and the initiation of treatment, the greater the recovery of motor regression and the gain of new motor acquisitions. The study found that the transition from "sitters" to "walkers" was positively correlated with the timing of the treatment initiation.

It's important to note that SMA type 2 patients tend to have milder symptoms and better prognosis as compared to SMA type 1, but that doesn't mean that their treatment should be delayed, on the contrary, early intervention is crucial for all SMA types, as the disease primarily affects motor neurons, which control muscle movement, and the earlier the treatment is started, the better the chances of preserving the re-maining motor neurons and slowing down the progression of the disease.

Our study found that the timing of treatment initiation had a significant impact on the functional prognosis of SMA patients. Specifically, it was found that the younger the age at the initiation of treatment, the better the functional prognosis ( $p < 0.001$ ,  $r = -0.553$ ). This highlights the importance of early diagnosis and prompt initiation of treatment for SMA patients in order to achieve the best possible outcome.

Additionally, the study found that the amplitude of the CMAP at the initial moment (T0) was also a predictor of motor evolution. Specifically, it was found that the higher the CMAP at the initial moment (T0), the better the motor evolution, with patients becoming "walkers" from "sitters" ( $p < 0.001$ ,  $r = 0.600$ ). This suggests that patients with higher CMAP values at the start of treatment are more likely to experience a better motor evolution.

The study also found that there were differences in clinical evolution between patients with 2 copies of SMN2 and those with 3 and 4 copies of SMN2. It was found that the clinical evolution was more modest in the group of patients with 2 copies of SMN2, which corresponded to the amplitude of CMAP. In this group, extremely low amplitudes of CMAP were observed, which increased slightly with evolution. However, there was an exception in the group of 2 copies of SMN2, where one child who initially (T0) had an amplitude of CMAP 2.8 mV evolved the best clinically, reaching at T26 to be a walker with normal CMAP. In the group of patients with 3 and 4 copies of SMN2, those who started with higher amplitudes of CMAP had a better clinical evolution.

Overall, our study suggests that the CMAP is a predictive parameter of evolution in SMA, which together with the score on the motor scales can indicate the prognosis for SMA patients. This information can be useful for healthcare professionals in monitoring the progression of the disease and making treatment decisions for SMA patients.

#### 4. Materials and Methods

In our research, we observed a total of 60 participants, comprising of 29 individuals diagnosed with SMA type 1 and 31 with SMA type 2. The treatment with nusinersen was initiated between the years 2018 and 2020, and the patients were closely monitored until 2022. All the patients in our study had been genetically confirmed to have SMA 5q and were experiencing symptoms at the start of their treatment.

The SMA type 1 group was relatively homogeneous, characterized by "non-sitters", the onset of symptoms occurring between the ages of 1-3 months. The age at diagnosis range between 1 and 8 months. The average age at which the treatment began was between 5 and 8 months. The regression of symptoms was observed quickly, within days or weeks, and the first motor and electrophysiological evaluations were performed at the start of treatment.

The SMA type 2 group was more diverse, characterized by "sitters", with an onset of symptoms occurring between the ages of 8-12 months, but with different forms of the disease, from "weak" – 2.1 (Dubowitz) to extremely hypotonic axially, with minimal movements in the lower limbs, up to "strong" – 2.9 (Dubowitz) who stands up-right, but needs support to walk [53]. In this group of patients, the diagnosis was in most cases much delayed, between 4 and 36 months, they had significant motor regression, the onset of tendinous retractions, and a decrease in muscle strength. The age of initiation of nusinersen treatment was months/years from the onset of symptoms, between 0 and 161 months. The results of the motor scales and the CMAP were analyzed to determine the possible correlation between these parameters.

In order to evaluate the motor skills of the patients, functional scales were used that corresponded to the type of disease. For SMA type 1 patients, the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) was used [54–57], and for SMA type 2 patients, the Hammersmith Functional Motor Scale Expanded (HFMSSE) was used [57,58]. Additionally, an electrophysiological study (CMAP in the right ulnar nerve) was performed before treatment (T0) and every 4 months up to 26 months (T26) in order to monitor the progress of the patients.

Amplitude of CMAP was recorded using a 6-channel EMG Keypoint device. Pediatric gel electrodes were applied at the level of the hypothenar eminence with supra-maximal stimulation of the ulnar nerve at the distal level (50–90 mV). To ensure that the CMAP results were reliable and reproducible, the electrical stimulation was repeated 3 times. The procedure was non-invasive and did not require sedation. Prior to the procedure, informed consent was obtained from the parents of the patients.

The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the National University Center for Children's Neurorehabilitation "Dr. Nicolae Robanescu" with approval number 7465/01.10.2018. In accordance with our drug administration standards, data were collected during periodic evaluations, and all patients' parents signed informed permission forms.

The IBM SPSS Statistics 24 and Excel 2021 programs were utilized for statistical data processing. Data processing for the investigation of possible correlations between the obtained data was guided by two statistical indicators: statistical significance ( $p$ ) and Pearson's correlation coefficient ( $r$ ) [59,60].

## 5. Conclusions

Our study found that the clinical evolution of SMA patients is closely tied to the initiation of disease-modifying treatment. Specifically, it was found that the earlier the treatment is started, the better the chances of preserving the remaining motor neurons, slowing down the progression of the disease, and recovering motor regression. This emphasizes the importance of early diagnosis and prompt initiation of treatment for SMA patients in order to achieve the best possible outcome.

Additionally, the study found that the number of SMN2 copies and the CMAP amplitude are important biomarkers for the evolution of the disease. Specifically, it was found that patients with 2 copies of SMN2 had a more modest clinical evolution compared to those with 3 and 4 copies of SMN2, which corresponded to the amplitude of CMAP. Similarly, it was found that patients with higher CMAP amplitudes at the initial moment (T0) had a better motor evolution. These results suggest that the number of SMN2 copies and the CMAP amplitude can be useful biomarkers for healthcare professionals to monitor the progression of the disease and make treatment decisions for SMA patients.

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**Informed Consent Statement:** Consent to participate in the study was acquired from the parents of all of the children enrolled in the study. Written informed consent has been obtained from the patient(s) to publish this paper.



**Data Availability Statement:** The corresponding authors can provide with access to the data contained in this study upon re-quest.

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## References

1. Darras BT, Markowitz JA, Monani UR, Darryl C. Spinal muscular atrophies. *Neuromuscul Disord* infancy, childhood, Adolesc. 2015;117–45.
2. Axente M, Mirea A, Sporea C, Pădure L, Drăgoi CM, Nicolae AC, et al. Clinical and Electrophysiological Changes in Pediatric Spinal Muscular Atrophy after 2 Years of Nusinersen Treatment. *Pharmaceutics*. 2022;14(10):2074.
3. Jablonka S, Hennlein L, Sendtner M. Therapy development for spinal muscular atrophy: perspectives for muscular dystrophies and neurodegenerative disorders. *Neurol Res Pract*. 2022;4(1):1–32.
4. Survival of Motor Neuron 1; SMN1 [Internet]. OMIM® - Online Mendelian Inheritance in Man®. Available from: <https://www.omim.org/entry/600354?search=SMN1&highlight=smn1>
5. Chen X, Sanchis-Juan A, French CE, Connell AJ, Delon I, Kingsbury Z, et al. Spinal muscular atrophy diagnosis and carrier screening from genome sequencing data. *Genet Med*. 2020;22(5):945–53.
6. Survival of Motor Neuron 2; SMN2 [Internet]. OMIM® - Online Mendelian Inheritance in Man®. Available from: <https://www.omim.org/entry/601627?search=SMN1&highlight=smn1>
7. Farrar MA, Kiernan MC. The genetics of spinal muscular atrophy: progress and challenges. *Neurotherapeutics*. 2015;12(2):290–302.
8. Markowitz JA, Singh P, Darras BT. Spinal muscular atrophy: a clinical and research update. *Pediatr Neurol*. 2012;46(1):1–12.
9. Eggermann K, Gläser D, Abicht A, Wirth B. Spinal muscular atrophy (5qSMA): best practice of diagnostics, newborn screening and therapy. *Medizinische Genet*. 2020;32(3):263–72.
10. Muscular Dystrophy Association [Internet]. [cited 2021 May 17]. Available from: <https://www.mda.org/disease/spinal-muscular-atrophy/types>
11. Dubowitz V. Very severe spinal muscular atrophy (SMA type 0): an expanding clinical phenotype. *Eur J Paediatr Neurol* [Internet]. 1999;3(2):49–51. Available from: <http://europepmc.org/abstract/MED/10700538>
12. Keinath MC, Prior DE, Prior TW. Spinal Muscular Atrophy: Mutations, Testing, and Clinical Relevance. *Appl Clin Genet*. 2021;14:11.
13. Spinal Muscular Atrophy, Type I; SMA1 [Internet]. OMIM® - Online Mendelian Inheritance in Man®. Available from: <https://www.omim.org/entry/253300?search=SMA type 0&highlight=0 sma type>
14. Pechmann A, Langer T, Schorling D, Stein S, Vogt S, Schara U, et al. Evaluation of children with SMA Type 1 under treatment with nusinersen within the expanded access program in Germany. *J Neuromuscul Dis*. 2018;5(2):135–43.
15. Ryan MM. The use of invasive ventilation is appropriate in children with genetically proven spinal muscular atrophy type 1: the motion against. *Paediatr Respir Rev*. 2008;9(1):51–4.
16. Saladini M, Nizzardo M, Govoni A, Taiana M, Bresolin N, Comi GP, et al. Spinal muscular atrophy with respiratory distress type 1: clinical phenotypes, molecular pathogenesis and therapeutic insights. *J Cell Mol Med*. 2020;24(2):1169–78.
17. Van Der Heul AMB, Cuppen I, Wadman RI, Asselman F, Schoenmakers MAGC, Van De Woude DR, et al. Feeding and Swallowing Problems in Infants with Spinal Muscular Atrophy Type 1: An Observational Study. *J Neuromuscul Dis*. 2020;7(3):323–30.
18. LoMauro A, Banfi P, Mastella C, Alberti K, Baranello G, Aliverti A. A new method for measuring bell-shaped chest induced by impaired ribcage muscles in spinal muscular atrophy children. *Front Neurol*. 2018;9:703.
19. Hergersberg M, Glatzel M, Capone AT, Achermann S, Hagemann A, Fischer J, et al. Deletions in the spinal muscular atrophy gene region in a newborn with neuropathy and extreme generalized muscular weakness. *Eur J Paediatr Neurol*. 2000;4(1):35–8.

20. Aragon-Gawinska K, Daron A, Ulinici A, Vanden Brande L, Seferian A, Gidaro T, et al. Sitting in patients with spinal muscular atrophy type 1 treated with nusinersen. *Dev Med Child Neurol*. 2020;62(3):310–4.
21. Bach JR, Gupta K, Reyna M, Hon A. Spinal muscular atrophy type 1: prolongation of survival by noninvasive respiratory aids. *Pediatr Asthma Allergy Immunol*. 2009;22(4):151–62.
22. Al-Zaidy SA, Mendell JR. From Clinical Trials to Clinical Practice: Practical Considerations for Gene Replacement Therapy in SMA Type 1. *Pediatr Neurol* [Internet]. 2019;100:3–11. Available from: <https://www.sciencedirect.com/science/article/pii/S0887899418311639>
23. Spinal Muscular Atrophy, Type II; SMA2 [Internet]. OMIM® - Online Mendelian Inheritance in Man®. Available from: <https://www.omim.org/entry/253550?search=SMA type 0&highlight=0 sma type>
24. Kaufmann P, McDermott MP, Darras BT, Finkel R, Kang P, Oskoui M, et al. Observational study of spinal muscular atrophy type 2 and 3: functional outcomes over 1 year. *Arch Neurol*. 2011;68(6):779–86.
25. Strauss KA, Farrar MA, Muntoni F, Saito K, Mendell JR, Servais L, et al. Onasemnogene abeparvovec for presymptomatic infants with three copies of SMN2 at risk for spinal muscular atrophy: the Phase III SPR1NT trial. *Nat Med*. 2022;28(7):1390–7.
26. Spinal Muscular Atrophy, Type III; SMA3 [Internet]. OMIM® - Online Mendelian Inheritance in Man®. Available from: <https://www.omim.org/entry/253400?search=SMA type 0&highlight=0 sma type>
27. Farrar MA, Vucic S, Johnston HM, du Sart D, Kiernan MC. Pathophysiological insights derived by natural history and motor function of spinal muscular atrophy. *J Pediatr*. 2013;162(1):155–9.
28. Burr P, Reddivari AKR. *Spinal Muscle Atrophy*. 2020;
29. Souza PVS, Pinto W, Ricarte A, Badia BML, Seneor DD, Teixeira DT, et al. Clinical and radiological profile of patients with spinal muscular atrophy type 4. *Eur J Neurol*. 2021;28(2):609–19.
30. Fardeau M, Desguerre I. Diagnostic workup for neuromuscular diseases. *Handb Clin Neurol*. 2013;113:1291–7.
31. Dulac O, Lassonde M, Sarnat HB. Diagnostic workup for neuromuscular diseases. *Pediatr Neurol Part III*. 2013;1291.
32. Bartousek J, Hlustik P, Grenarova O, Beranova M. Advanced EMG techniques in diagnostics of spinal muscular atrophies and motor neuron disease—magnetic stimulation, single fiber EMG and macro EMG. *Acta Univ Palacki Olomuc Fac Med*. 1993;136:37–9.
33. Rudnik-Schöneborn S, Goebel HH, Schlote W, Molaian S, Omran H, Ketelsen U, et al. Classical infantile spinal muscular atrophy with SMN deficiency causes sensory neuropathy. *Neurology*. 2003;60(6):983–7.
34. Arkblad EL, Darin N, Berg K, Kimber E, Brandberg G, Lindberg C, et al. Multiplex ligation-dependent probe amplification improves diagnostics in spinal muscular atrophy. *Neuromuscul Disord*. 2006;16(12):830–8.
35. Targeted qPCR SMA for Spinal Muscular Atrophy Newborn Screening [Internet]. [cited 2023 Jan 10]. Available from: <https://www.zentech.be/en/spinal-muscular-atrophy-newborn-screening-test>
36. D'Amico A, Mercuri E, Tiziano FD, Bertini E. Spinal muscular atrophy. *Orphanet J Rare Dis* [Internet]. 2011;6(1):71. Available from: <https://doi.org/10.1186/1750-1172-6-71>
37. Mishra VN, Kalita J, Kesari A, Mitta B, Shankar SK, Misra UK. A clinical and genetic study of spinal muscular atrophy. *Electromyogr Clin Neurophysiol*. 2004;44(5):307–12.
38. Arnold WD, Kassir D, Kissel JT. Spinal muscular atrophy: Diagnosis and management in a new therapeutic era. *Muscle and Nerve*. 2015;51(2):157–67.
39. Lewelt A, Krosschell KJ, Scott C, Sakonju A, Kissel JT, Crawford TO, et al. Compound muscle action potential and motor function in children with spinal muscular atrophy. *Muscle Nerve*. 2010 Nov;42(5):703–8.
40. Mirea A, Shelby E-S, Axente M, Badina M, Padure L, Leanca M, et al. Combination Therapy with Nusinersen and Onasemnogene Abeparvovec-xioi in Spinal Muscular Atrophy Type I. *J Clin Med*. 2021;10(23):5540.
41. Sleutjes BTHM, Wijngaarde CA, Wadman RI, Otto LAM, Asselman F-L, Cuppen I, et al. Assessment of motor unit loss in patients with spinal muscular atrophy. *Clin Neurophysiol*. 2020;131(6):1280–6.
42. Kariyawasam DST, D'silva A, Lin C, Ryan MM, Farrar MA. Biomarkers and the development of a personalized medicine approach in spinal muscular atrophy. *Front Neurol*. 2019;10(AUG):1–12.
43. European Medicines Agency. Annex 1. Summary of Product Characteristics [Internet]. European Public Assessment Report for Spinraza. [cited 2021 May 17]. Available from: [https://www.ema.europa.eu/en/documents/product-information/spinraza-epar-product-information\\_en.pdf](https://www.ema.europa.eu/en/documents/product-information/spinraza-epar-product-information_en.pdf)

44. American Society of Health-System Pharmacists. Spinraza (nusinersen) Approved [Internet]. American Society of Health-System Pharmacists Clinical Drug Information. [cited 2021 May 17]. Available from: <https://www.ahfsdruginformation.com/spinraza-nusinersen-approved/>
45. European Medicines Agency. EU/3/12/976: Orphan designation for the treatment of 5q spinal muscular atrophy [Internet]. Available from: <https://www.ema.europa.eu/en/medicines/human/orphan-designations/eu312976>
46. Hua Y, Sahashi K, Hung G, Rigo F, Passini MA, Bennett CF, et al. Antisense correction of SMN2 splicing in the CNS rescues necrosis in a type III SMA mouse model. *Genes Dev.* 2010;24(15):1634–44.
47. Biogen. Intrathecal administration with SPINRAZA® (nusinersen) [Internet]. [cited 2023 Oct 1]. Available from: [https://www.spinraza.com/content/dam/commercial/spinraza/caregiver/en\\_us/pdf/Understanding-Intrathecal-Administration.pdf](https://www.spinraza.com/content/dam/commercial/spinraza/caregiver/en_us/pdf/Understanding-Intrathecal-Administration.pdf)
48. Figueiredo M. Motor Function Improved After 1 Year of Spinraza in Children with SMA Types 1–2 [Internet]. BioNews. SMA News Today. [cited 2021 May 17]. Available from: <https://smanewstoday.com/news-posts/2020/06/24/spinraza-improves-motor-function-after-1-year-children-sma-type-1-2-real-life-study-france/>
49. Figueiredo M. Spinraza (Nusinersen) [Internet]. BioNews. SMA News Today. Available from: <https://smanewstoday.com/spinraza-nusinersen/>
50. LoMauro A, Mastella C, Alberti K, Masson R, Aliverti A, Baranello G. Effect of nusinersen on respiratory muscle function in different subtypes of type 1 spinal muscular atrophy. *Am J Respir Crit Care Med.* 2019;200(12):1547–50.
51. Darras BT, Farrar MA, Mercuri E, Finkel RS, Foster R, Hughes SG, et al. An Integrated Safety Analysis of Infants and Children with Symptomatic Spinal Muscular Atrophy (SMA) Treated with Nusinersen in Seven Clinical Trials. *CNS Drugs* [Internet]. 2019;33(9):919–32. Available from: <https://doi.org/10.1007/s40263-019-00656-w>
52. Biogen. Dosing designed to give your body the medicine it needs, when it needs it. [Internet]. [cited 2023 Oct 1]. Available from: [https://www.spinraza.com/en\\_us/home/taking-spinraza/dosing.html](https://www.spinraza.com/en_us/home/taking-spinraza/dosing.html)
53. Dubowitz V. Sixty years of spinal muscular atrophy: A personal odyssey. Sumner CJ, Paushkin S Ko C Spinal Muscular Atrophy Dis Mech Ther pp xvii–xxi. 2016;
54. Kichula E, Duong T, Glanzman A, Pasternak A, Darras B, Finkel R, et al. Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) Feasibility for Individuals with Severe Spinal Muscular Atrophy II (S46. 004). AAN Enterprises; 2018.
55. Glanzman AM, Mazzone E, Main M, Pelliccioni M, Wood J, Swoboda KJ, et al. The Children’s Hospital of Philadelphia infant test of neuromuscular disorders (CHOP INTEND): test development and reliability. *Neuromuscul Disord.* 2010;20(3):155–61.
56. Spinal Muscular Atrophy UK. CHOP INTEND (Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders) [Internet]. [cited 2022 Nov 28]. Available from: <https://smauk.org.uk/chop-intend>
57. Measures of mobility and physical ability evaluated in clinical trials [Internet]. [cited 2022 Nov 28]. Available from: [https://www.spinraza-hcp.com/content/dam/commercial/spinraza/hcp/en\\_us/pdf/mobility-and-physical-ability.pdf](https://www.spinraza-hcp.com/content/dam/commercial/spinraza/hcp/en_us/pdf/mobility-and-physical-ability.pdf)
58. Pera MC, Coratti G, Forcina N, Mazzone ES, Scoto M, Montes J, et al. Content validity and clinical meaningfulness of the HFMSE in spinal muscular atrophy. *BMC Neurol.* 2017;17(1):1–10.
59. StatisticsSolutions. Pearson’s Correlation Coefficient [Internet]. [cited 2022 Sep 30]. Available from: <https://www.statisticssolutions.com/pearsons-correlation-coefficient/>
60. Williams F, Monge P. Reasoning with statistics: How to read quantitative research. 5th Editio. Orlando, Florida, USA: Harcourt College Publishers; 2001. 228 p.