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Impact of Nusinersen on Neurofilament, Creatinine Levels, and Motor Function in Pediatric Spinal Muscular Atrophy Rehabilitation: A Biomarker Analysis

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Abstract: Background: Spinal amyotrophy is a rare, neurodegenerative disease, with progressive evolution, disabling until death in severe forms, but for which 3 disease-modifying drugs have recently been approved (in the last 8 years). In this context, it became necessary to find predictive factors for the evolution of patients and for the effectiveness of the treatment applied to personalize the therapy to obtain the best results according to the particularities of each patient. **Objective:** The objective of this retrospective study is to analyze the evolution of different clinical (motor functional scales) and paraclinical biomarkers (level of pNF-H neurofilaments in serum and cerebrospinal fluid and of serum creatinine) under treatment with nusinersen in various types of spinal muscular atrophy (SMA). **Methods**: We analyzed the biomarkers values for a group of 69 pediatric patients diagnosed with SMA in different stages of treatment over three years, depending on the type of SMA, the number of copies of the SMN2 gene, and the age at initiation of therapy. **Results**: We observed significant increases in the levels of pNF-H neurofilaments in both cerebrospinal fluid (CSF) and serum, with correlations to the age of symptom onset in patients and an inverse relationship to the number of copies of the SMN2 gene. These levels decreased during treatment with nusinersen, coinciding with increased serum creatinine values and improved motor functional assessment scores. The most pronounced effects were noted in patients with severe forms of the disease, such as SMA type 1, mainly when treatment was initiated at a younger age. **Conclusion**: The evolution of patients under disease-modifying treatments should be analyzed both for the evolution on specific motor functional scales, as well as against the biomarkers of neuronal degradation: pNF-H, present in CSF and serum, and serum creatinine, a marker of muscle activity. Administering the disease-modifying treatment promptly after diagnostic confirmation halts neural degradation and enhances the patient's motor function.

Keywords: spinal muscular atrophy; neurofilaments; cerebrospinal fluid; biomarkers; nusinersen; creatinine; motor evolution

1. Introduction

Spinal muscular atrophy (SMA), one of the most common neurodegenerative diseases in the pediatric population, is a genetic disease determined in more than 95% of

cases by mutations in the SMN1 gene, which encodes the SMN protein necessary for the survival of motor neurons, a gene located in the 5q13 region [1,2].

In the same region, there is a similar gene, the SMN2 gene, which differs from the first one by replacing a single amino acid – C with T, in exon 7 at codon 280, a change sufficient to alter mRNA spicing and thus synthesizes about 10% of the normal SMN protein, the rest being a structurally and functionally modified protein, rapidly degradable [3,4].

Clinical manifestations depend on the number of copies of the SMN2 gene present and range from progressive muscle weakness and atrophy to respiratory and digestive manifestations [5–7].

SMA is a rare disease with a prevalence of 1-2 per 100,000 population and an incidence of 1 per 10,000 live births. Before the advent of disease-modifying treatments, it was classified by the age of onset of symptoms, prognosis, and stage of motor skills development into five types, which were sub-classified into sub-types, depending on their severity [8,9].

Patients with SMA type 0, which is exceedingly rare, typically possess only one copy of the SMN2 gene. Manifestations of the disease are evident from intrauterine life, often necessitating ventilatory support at birth. Life expectancy for individuals with SMA type 0 is typically limited to several months [10,11].

Of all cases of the disease in the pediatric population, approximately 58% are SMA type 1, 29% type 2, and 13% type 3 [12,13]. In type 1, the manifestations appear in the first six months of life, 2 copies of the SMN2 gene are present in the offspring, the children fail to acquire walking, and the average life expectancy is 13.5 months [6,14]. In type 2, with the onset of symptoms after 6 months, patients can sit but do not acquire the ability to walk independently and generally have 2 or 3 copies of the SMN2 gene [6,14]. Type 3 patients show symptoms after 2 years but gradually lose walking ability [6]. Type 4 is specific to adulthood, and it is the mildest SMA form. It evolves with diffuse muscle weakness and a decrease in the amplitude of deep tendon reflexes. However, patients can move, the disease causes no digestive or respiratory conditions, and life expectancy is unaffected.

Currently, three types of treatments are approved to increase the amount of SMN available in the body. These treatments vary in their mode of action, administration route, and tissue level bioavailability [15–17].

The first drug approved by the US Food and Drug Administration (FDA) in 2016 and then by the European Commission for the treatment of SMA was nusinersen, an antisense oligonucleotide administered intrathecally, following a loading and maintenance schedule, targeting the messenger RNA of the SMN2 gene, modifying its splicing and thus causing the production of an increased amount of SMN with typical structure [18,19]. Onasemnogene abeparvovec, approved in 2020 by the FDA, is based on gene therapy, with a single intravenous administration of a DNA virus associated with the SMN gene [20]. Risdiplam, the third approved one in 2021, a daily oral small-molecule drug, also acts on the SMN2 pre-messenger RNA to increase the amount of functional SMN protein by altering gene splicing [8,21].

In neuromuscular diseases, the degree of physical damage, the progression of the disease, the clinical evolution, and the effectiveness of the treatment are established on quantitative scales designed to measure motor function. Dedicated scales have been developed for spinal amyotrophy, according to different types of disease, and correlated with the severity of symptoms [22,23]. The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) is a motor skills assessment scale created specifically for children diagnosed with spinal amyotrophy type 1, non-sitters, and evaluates the strength of the axial and peripheral muscles [8,24]. The scale has a maximum of 64 points from the evaluation of 16 items in 4 different degrees of difficulty (from 0 to 4) addressing spontaneous movements, hand grip, head stabilization, flexion, and extension of the limbs [8]. The Expanded Hammersmith Functional Motor Scale (HFMSE), recommended for children over 2 years old, sitters, and walkers, is focused on assessing

gross motor function in SMA types 2 and 3 patients [8]. HFMSE is a 33-item test with a score between 0 and 2 points, with a maximum of 66 points.

Serum creatinine has proven to be a biomarker to consider for tracking the progressive denervation that occurs in SMA, with the decrease in this parameter's serum level correlating with the severity of the disease [25,26].

Creatinine is formed at the muscle level by metabolizing phosphocreatine to release the energy stored at this level. The correlation of the serum level of creatinine with the severity and progression of the disease has also been studied in other neuromuscular diseases (for example, Kennedy's disease, Becker or Duchenne muscular dystrophy). It is more helpful in assessing muscle function than creatine kinase, which assesses the degree of muscle damage. However, the degree of correlation was less sensitive [27,28].

Neurofilaments are exclusively neuronal proteins, studied more and more in recent years as possible biomarkers of neuronal aggression and degradation due to the increased concentration in serum and CSF in various acute neuronal conditions and neurodegenerative diseases like Alzheimer's disease, dementia with Lewy bodies, Parkinson's disease, frontotemporal dementia, amyotrophic lateral sclerosis, Charcot-Marie-Tooth disease, multiple sclerosis, toxic or giant axon neuropathy [29,30]. They are polymers belonging to class IV of intermediate filaments due to their diameter of 10 nm, located between that of microfilaments (7 nm) and microtubules (25 nm), together with which they form the neuronal cytoskeleton [29,30].

Researchers have differentiated five types of neurofilaments based on physical, chemical, and, more recently, genetic criteria. Three types—neurofilaments low (NF-L), medium (NF-M), and high (NF-H)—are present at both central and peripheral levels and are named according to their molecular weight. A fourth subunit is added – α-internexin, in the central nervous system, and peripherin, in the peripheral nervous system added to these neurofilaments [30–33]. The five subunits share a core domain with an αhelix structure, flanked by terminal amino and carboxy domains that regulate interactions with other molecules and polymerization [29].

At the level of the terminal domains, there is a variable number of lysine, serine, threonine, or other sites where the biochemical processes of nitration, glycosylation, oxidation, ubiquitylation, and phosphorylation take place necessary for the exercise of functions or the transport of neurofilaments, from the level of the neuronal body - where the synthesis of neurofilaments takes place, along the axons - where they mainly carry out their activity [34–36].

pNF-H, the phosphorylated form of the largest subunit, is found in the most significant amount at the axonal level. Due to intense phosphorylation, pNF-H exhibits a high degree of stability against the action of proteolytic enzymes [36–38].

Diseases that develop with damage or destruction of neurons lead to the release of increased amounts of neuronal components and implicitly to the appearance of pNF-H in the interneuronal space, from where they reach the cerebrospinal fluid and then the serum [30,39,40].

For the pediatric population with SMA, the highest levels of pNF-H were observed in patients without specific treatment. CSF levels were significantly higher among patients with SMA type 1 than serum levels [41].

The decrease in the increased levels of pNF-H in the blood of patients with SMA type 1 during treatment with nusinersen supports using this biomarker to monitor the effects of reducing neuronal degeneration in the pediatric population [42,43].

In the studies on adult patients with SMA type 2 and 3 for the evaluation of the use of neurofilaments for monitoring the evolution under treatment with nusinersen, the change in the level of the CSF will not have been as spectacular as in the pediatric population, probably due to the low reserve of neurons that can be affected by degeneration processes [42,44,45]. The decrease in the pNF-H level in a disease with progressive evolution was due to the specific disease-modifying treatment. However, in forms with long evolution, these changes correlate too little with favorable results on the functional motor scales [44].

The objectives of this study were to analyze the changes in the level of pNF-H neurofilaments in the cerebrospinal fluid and the serum during treatment with nusinersen for spinal amyotrophy types 1, 2, and 3 in the pediatric population, compared to the values obtained at the start of the treatment and the beginning of the maintenance period. In addition, the relationship between changes in CSF pNF-H level compared to changes in serum - as markers of neuronal degradation, as well as to serum creatinine - a marker of muscle atrophy and scores obtained on motor assessment scales - as evidence objective of motor performance changes.

2. Materials and Methods

This retrospective study was conducted on patients treated in the National Teaching Center for Children's Neurorehabilitation "Dr. Nicolae Robanescu" between November 2020 and November 2023 for spinal amyotrophy with nusinersen - the disease-modifying antisense oligonucleotide. All available data of pairs of samples - serum and CSF, collected just before intrathecal administration of the drug, at different stages of the treatment (before initiation - the first dose, before the start of maintenance treatment - the fifth dose, one, two or three years after the initiation of therapy – doses 7, 10, and 13 respectively of the drug).

The study was approved by the hospital's Ethics Committee (agreement no. 7464/01.10.2018). Informed consent to participate was obtained from the patient's legal relatives following local regulations and the Declaration of the World Medical Association of Helsinki, revised in 2000 in Edinburgh.

The samples analyzed in this study were obtained as part of the standard protocol for patient evaluation, involving the collection of blood samples for recommended tests before injection, and CSF collection just prior to treatment administration, following the manufacturer's instructions. No supplementary maneuvers were required for sample collection.

The inclusion criteria were: pediatric patient at the time of treatment initiation (under 18 years old), included in the national treatment program with nusinersen, with the existence of a compound homozygous or heterozygous mutation (deletion) of the SMN1 gene in the 5q region confirmed by genetic analysis and the existence of at least 2 copies of the SMN2 gene, without other disease-modifying treatments, with the existence of at least two pairs of serum-CSF samples collected before administration of doses 1, 5, 7, 10 and 13 of treatment and adequately preserved for analysis laboratory and with the evaluations on the motor scales corresponding to the analyzed data.

The following situations were considered exclusion criteria for participation in this study: lack of serum or cerebrospinal fluid samples suitable for pNF-H analysis, the existence or appearance of other conditions that evolve with neuronal damage and could increase the number of neurofilaments, conditions that interfere with the way of cerebrospinal fluid circulation or the additional administration of another specific disease-modifying treatment.

To determine the level of pNF-H, we used the enzyme immunoassay (ELISA) technique for a quantitative in vitro determination of phosphorylated neurofilament heavy chain (pNF-H). Based on the reaction between the polyclonal anti-pNF-H antibodies with which the reaction wells are coated and the complex formed by the anti-pNF-H monoclonal antibodies labeled with peroxidase with which the CSF or serum samples were initially treated, the test measures the color intensity, proportional to the amount of pNF-H neurofilaments in the samples. The pre-analytical processing, sample preservation, and working method followed the instructions of the reagent kit manufacturer — EUROIMMUN Medizinische Labordiagnostika AG (Seekamp 31, 23560 Lübeck, Germany) — with the help of the ELISA BIORAD 3100 PSC microplate reader (Bio-Rad Laboratories, 1000 Alfred Nobel Drive, Hercules, CA 94547, USA). Following tests performed on ALS patients and control groups, the manufacturer obtained a sensitivity of 93.7% for CSF samples at a specificity of 93.9%, and for serum samples, the

sensitivity was 93.3% at a specificity of 92.7% using this kit of reagents for the in vitro diagnosis of ALS.

Blood samples were collected for laboratory analyses after the pediatric and neurological consultation to perform the puncture for administering nusinersen and after assessing motor functions on the functional scales specially designed for patients with spinal amyotrophy. After collection in specific vials with a separator clot, the samples were centrifuged for 15 minutes at 3600 rpm. The separated serum was analyzed from the point of view of macroscopic appearance (not to be hemolyzed or icteric), and the creatinine level of the sample was determined. It was preserved at temperatures below -20°C to determine pNF-H neurofilaments. Creatinine determination was performed by the improved Jaffe method, based on the reaction of creatinine with sodium picrate, on the Arhitect c4100 analyzer (Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, USA).

Cerebrospinal fluid was collected during lumbar puncture for the administration of nusinersen treatment, just before drug injection, in a sterile container and aliquoted into two samples. The first sample was visually analyzed for macroscopic appearance regarding turbidity and color. Turbidity was assessed as a clear liquid equivalent to 0 on the McFarland scale, respectively cloudy liquid for any other value. For color, a 4-step scale was used, from 0 – corresponding to a colorless liquid, 1 – for a liquid with traces of pink, 2 – a liquid with more pronounced traces of pink, and up to 3 – a liquid with a hemorrhagic appearance.

Cerebrospinal fluid cellularity was determined in the Bürker–Türk (Fischer Scientific Company L.l.c., P.O. Box 1768, Pittsburgh, PA 15275, USA) counting chamber by calculating the number of nucleated elements present per cubic millimeter of fluid, with a Nikon Eclipse E 100/ 300 microscopes (Nikon, Minato City, Tokyo, Japan). Chlorine content was determined by the potentiometric method using the ionometer I Smart-30 Pro (i-Sens, Inc., Seoul, Republic of Korea). The second sample was preserved at temperatures below -20°C to determine pNF-H neurofilaments, according to the reagent kit manufacturer's instructions.

The patients' demographic, anthropometric, and clinical data were collected from the observation sheets and the computer system following pediatricians' and neurologists' consultations. Physical therapists specializing in evaluating children with SMA determined motor scale scores to establish functional performance. The paraclinical data resulted from the processing of the samples by qualified medical personnel and the analysis and validation of the results by laboratory doctors.

Statistical analyses were conducted using IBM SPSS Statistics 24 and Microsoft Excel 2021 after verifying the normality of distributions with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive analyses of the dataset considered mean values ± SD for normally distributed data and median for non-normally distributed data. We analyzed the potential differences between the biomarkers and the motor scores assessed among patient groups categorized by type of SMA and, respectively, the number of SMN2 copies using the ANOVA test. For groups where differences were identified, a post hoc analysis was conducted. Additionally, paired sample t-tests were performed, and correlations between variables were assessed using Pearson correlation. Test results with $p < 0.05$ were considered statistically significant.

3. Results

Our study included 69 patients from whom 304 data sets containing pNF-H levels in serum and cerebrospinal fluid were analyzed. The youngest patient, aged 20 days at the time of initiation of treatment, a male, was diagnosed with SMA type 1 and had 2 copies of the SMN2 gene. The oldest patient was also male, aged 15 and five months, with SMA type 3 and 3 copies of the SMN2 gene. Table 1 displays the characteristics of the group based on participant age at the initiation of treatment, sex, SMA type, and number of SMN2 copies.

Table 1. Characteristics of the study group participants

		SMA			No of SMN2 copies	
	All types	**Type 1**	**Type 2**	**Type 3**	**2**	**3**	**4**
No	69	31	26	12	30	36	3
Sex – Male	32	15	13	4	11	20	1
Age – months							
Min ÷ Max	0.67÷185	0.67÷86	13÷177	30÷185	0.67÷176	5÷185	30÷114
Mean ± SD/ Median	52.90±57.97	12.94±20.20	73.62±59.20	112	28.44±42.18	71.58±63.62	76
SMN Copies (No of cases)							
2 copies		22	4	4			
3 copies		9	19	8			
4 copies		-	3	-			

Table 2 presents the minimum and maximum limits, the mean or median of pNF-H levels in CSF and serum, serum creatinine, and the scores obtained in the functional evaluations categorized by SMA type and the number of SMN2 copies at treatment initiation.

Table 2. Summary of Biomarkers and Functional Evaluation Scores by SMA Type and SMN2 Copy Number at Treatment Initiation

		SMA			No of SMN2 copies	
	All types	**Type 1**	**Type 2**	**Type 3**	**2**	**3**	**4**
pNF-H in CSF (ng/ml)							
Min ÷ Max	0.035÷2.424	0.039÷2.424	0.035÷0.817	0.04÷0.386	0.039÷2.424	0.035÷0.817	
Mean ± SD/ Median	0.409±0.562	0.713±0.764	0.214±0.20	0.156	0.685±0.758	0.203±0.179	0.154
pNF-H in serum (ng/ml)							
Min ÷ Max	0.032÷5.268	0.032÷4.595	0.212÷5.268	0.05÷1.982	0.05÷4.595	0.032÷5.268	
Mean ± SD/ Median	1.298±1.495	1.318±1.401	1.583±2.131	0.137	0.773	1.05±1.419	
Creatinine (mg/dl)							
Min ÷ Max	0.22÷0.62	0.22÷0.44	0.25÷0.52	0.24÷0.62	0.22÷0.62	0.24÷0.52	
Mean ± SD/ Median	0.352±0.073	0.355	0.334±0.072	0.360	0.358±0.787	0.36	0.25
MFM/ CHOP							
Min ÷ Max	7÷57	7÷57			7÷50	24÷57	
Mean ± SD/ Median	24	24			23.76±11.755	37.50	
MFM/ HFMSE							
Min ÷ Max	0÷54		0÷34	16÷54	7÷54	0÷53	
Mean ± SD/ Median	20		16	36.5	37	20	10

Following disease-modifying treatments, the classification of SMA changed based on motor performance, with many patients transitioning from non-sitters to sitters and then to walkers. At the beginning of treatment, patients were initially categorized based on the type of SMA and the number of SMN2 gene copies to enable comparison and objective monitoring over time using these two criteria. Additionally, to account for differences in evaluation scales and individual treatment response, changes in scale performance between evaluation moments and relative changes in pNF-H levels compared to initial and previous values were considered.

3.1. pNF-H level in CSF

At baseline, the highest CSF pNF-H value was 2.424 ng/mL, observed in a 2.57-month-old male patient with SMA type 1 and 2 copies of the SMN2 gene. The lowest value recorded was 0.035 ng/mL, found in a 104-month-old male patient at initiation with SMA type 2 and 3 copies of the SMN2 gene.

Following the loading period, just before the commencement of maintenance treatment, the highest value—0.772 ng/mL—was observed in a male patient aged 172 months at the start, diagnosed with type 2 SMA and possessing 3 copies of the SMN2 gene. Conversely, the lowest value—0.009 ng/mL—was recorded in two patients with SMA type 1. One patient, a 10-month-old male at initiation, had 3 copies of the SMN2 gene, while the other, a female, was 6.5 months old at initiation and possessed 2 copies of the SMN2 gene.

Figures 1 and 2 present the evolution of pNF-H levels in CSF, categorized by SMA type and number of SMN2 copies, at analyzed time moments.

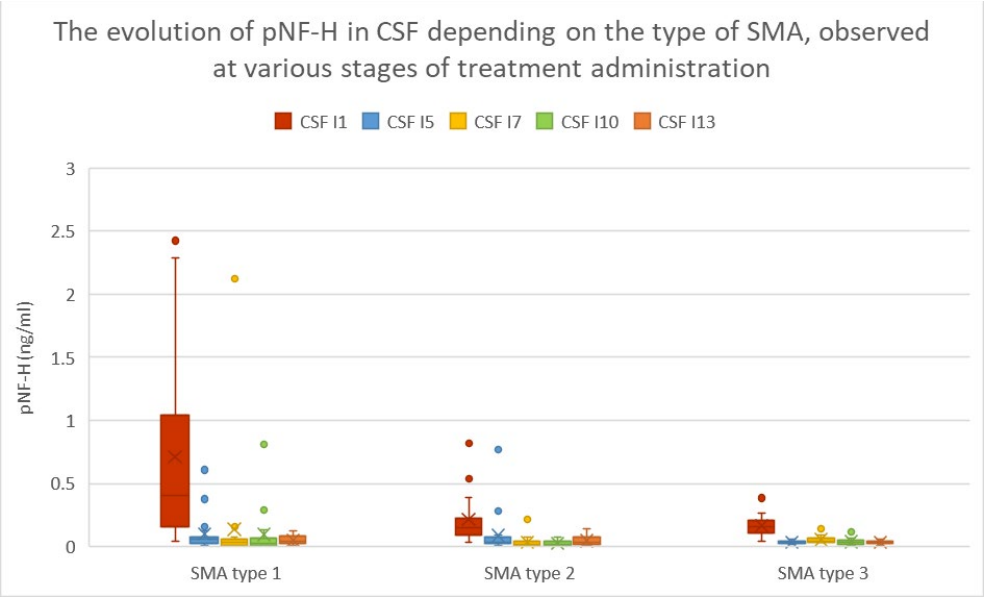

Figure 1. The evolution of pNF-H in CSF, according to SMA type, at different moments of the treatment (I1- at the initiation of therapy, I5 - before the start of maintenance treatment, I7 - at one year of therapy, I10 – at two years of treatment, and I13 – at three years of treatment)

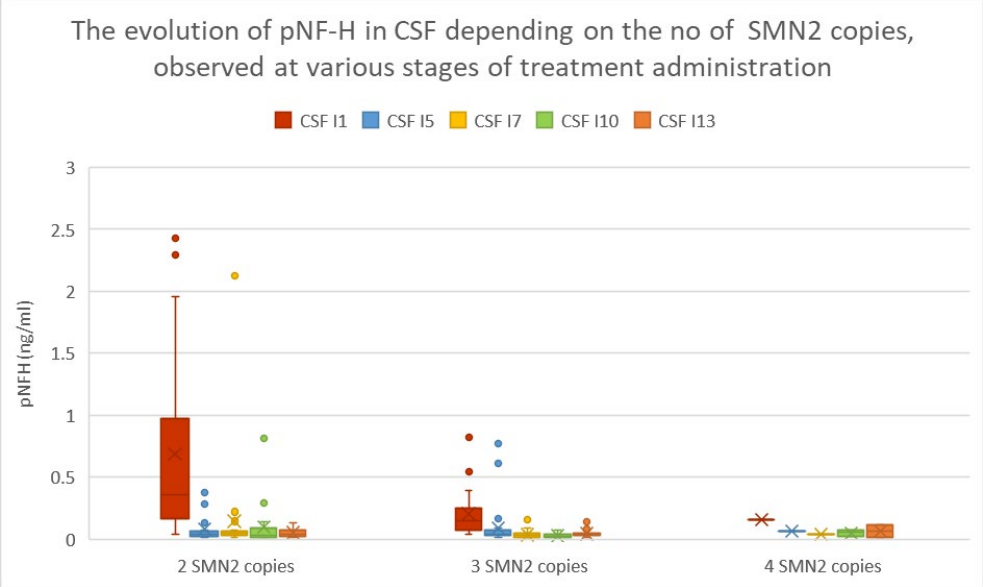

Figure 2. The evolution of pNF-H in CSF, according to the number of SMN2 copies, at different moments of the treatment (I1- at the initiation of therapy, I5 - before the start of maintenance treatment, I7 - at one year of therapy, I10 – at two years of treatment, and I13 – at three years of treatment)

3.2. pNF-H level in serum

The lowest serum pNF-H values at the initiation and the beginning of maintenance treatment were observed in two female patients with SMA type 1. One patient had 3 SMN2 copies, with a serum pNF-H level of 0.032 ng/mL at initiation and was 10 months old, while the other had 2 SMN2 copies, with a serum pNF-H level of 0.009 ng/mL at initiation and was 5.03 months old.

On the contrary, the maximum serum pNF-H values at these specific time points were recorded in two male patients. One patient, diagnosed with SMA type 2 and possessing 3 SMN2 copies, had a serum pNF-H level of 5,268 ng/mL at 17 months of age at the start of treatment. The other patient, diagnosed with SMA type 1 and possessing 2 SMN2 copies, had a serum pNF-H level of 7.28 ng/mL at 4 months of age at the start of treatment.

Figures 3 and 4 present the evolution of pNF-H levels in serum, categorized by SMA type and number of SMN2 copies, at analyzed time moments.

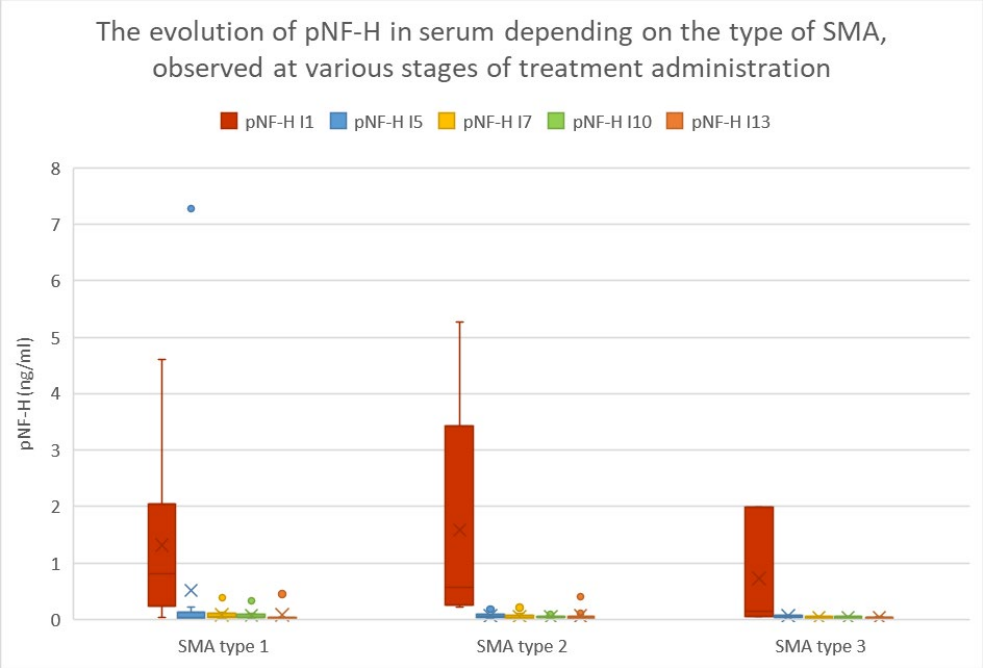

Figure 3. The evolution of pNF-H in serum, according to SMA type, at different moments of the treatment (I1- at the initiation of therapy, I5 - before the start of maintenance treatment, I7 - at one year of therapy, I10 – at two years of treatment, and I13 – at three years of treatment)

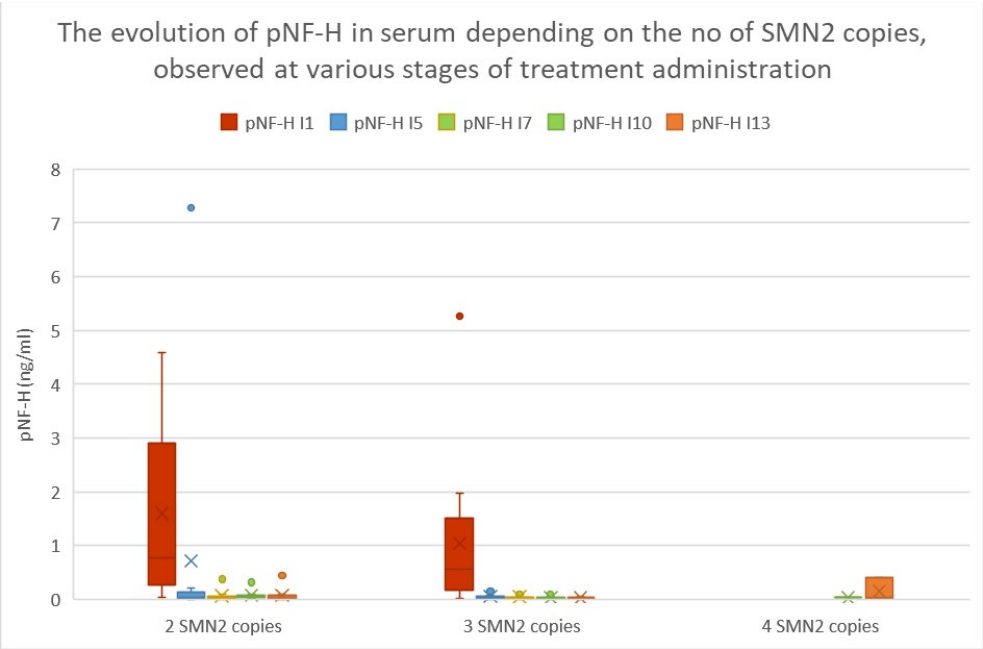

Figure 4. The evolution of pNF-H in serum, according to the number of SMN2 copies, at different moments of the treatment (I1- at the initiation of therapy, I5 - before the start of maintenance treatment, I7 - at one year of therapy, I10 – at two years of treatment, and I13 – at three years of treatment)

3.3. Serum creatinine level

Regarding serum creatinine values, the lowest and highest values at the initiation of treatment and the beginning of the maintenance period were observed in four female patients.

At baseline, the highest serum creatinine value was 0.62 mg/dL, observed in a 176-month-old patient with SMA type 3 and 2 copies of the SMN2 gene. Conversely, the lowest value, 0.22 mg/dL, was recorded in a patient aged 6.57 months with SMA type 1 and 2 copies of the SMN2 gene.

Before the start of maintenance treatment, the highest value observed was 0.72 mg/dL in a patient who was 6.3 months old at initiation, diagnosed with SMA type 1, and possessing 2 SMN2 copies. The lowest value was observed in a patient who was 150 months old at treatment initiation, with SMA type 3 and 3 SMN2 copies.

Figures 5 and 6 present the evolution of serum creatinine, categorized by SMA type and number of SMN2 copies, at analyzed time moments.

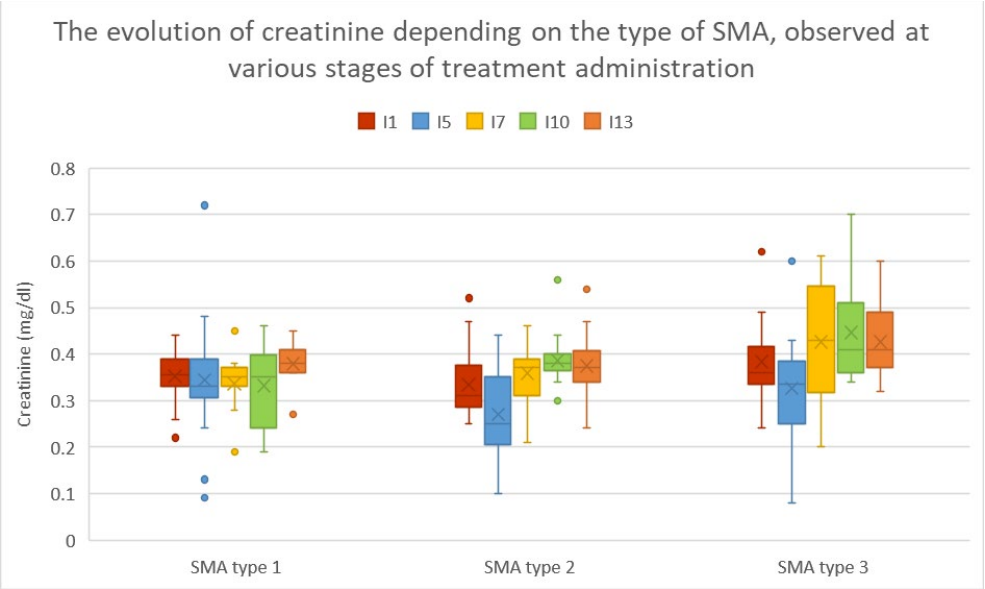

Figure 5. The evolution of creatinine according to SMA type at different moments of the treatment (I1- at the initiation of therapy, I5 - before the start of maintenance treatment, I7 - at one year of therapy, I10 – at two years of treatment, and I13 – at three years of treatment)

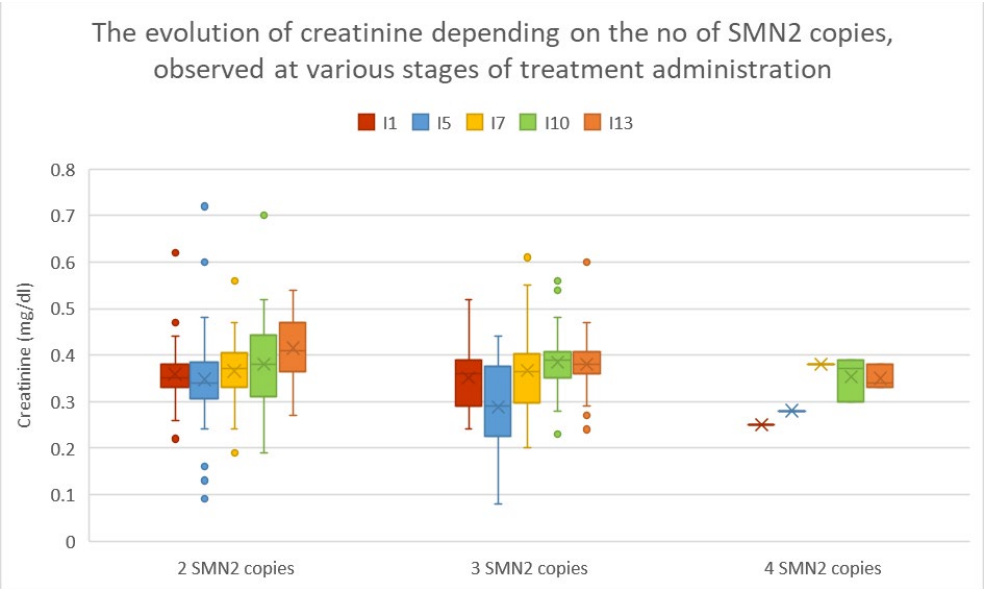

Figure 6. The evolution of creatinine according to the number of SMN2 copies at different moments of the treatment (I1- at the initiation of therapy, I5 - before the start of maintenance treatment, I7 - at one year of therapy, I10 – at two years of treatment, and I13 – at three years of treatment)

3.4. Motor function scores

The assessment of motor performance included the CHOP INTEND scale for patients with SMA type 1 and the HFMSE scale for patients diagnosed with SMA type 2 and 3. The results obtained at the initiation of treatment are presented in Table 2.

The lowest score on the CHOP INTEND scale at the start of treatment was 7 points, recorded in a 0.67-month-old boy with 2 copies of the SMN2 gene, while the highest score was 57 points, achieved by a 10-month-old boy with 3 copies of the SMN2 gene. Before commencing maintenance treatment, the lowest score was 21 points, observed in a 76-month-old girl at initiation, also with 2 SMN2 copies. In comparison, the highest score was 64 points and was attained at treatment initiation by a 1.3-month-old girl with 2 copies of the SMN2 gene.

On the HFMSE scale, both at the beginning and before the start of maintenance treatment, the lowest values (0 points) were recorded in patients with SMA type 2 and 3 copies of the SMN2 gene. In comparison, the highest values (54 and 63 points, respectively) were observed in patients with SMA type 3 and 2 copies of the SMN2 gene.

Two patients, one male, aged 177 months, and one female, aged 164 months, received a score of 0 points at the initiation of treatment, with the same patient receiving the same score before starting maintenance treatment. The highest value at initiation was 54 points, achieved by two patients: a 50-month-old boy and a 176-month-old girl. The same girl attained the highest score of 63 points at the beginning of the maintenance treatment.

Figures 7 and 8 illustrate how motor functioning, represented as yield, changes over time based on patient performance compared to their initial evaluation. This data is categorized by SMA type and the number of SMN2 gene copies, which are crucial factors in understanding the progression and severity of SMA.

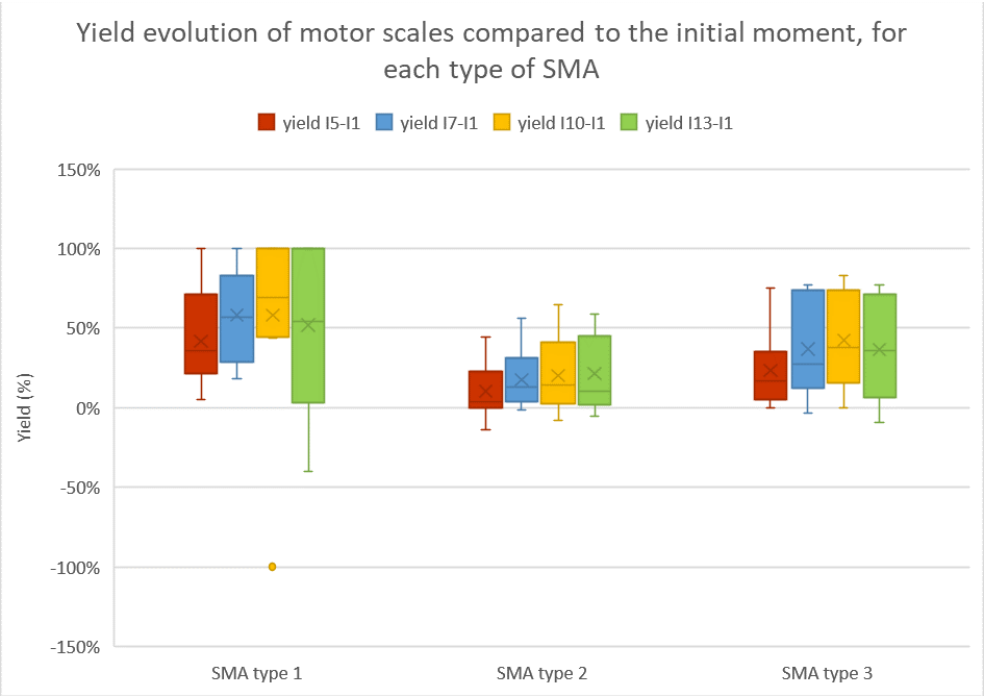

Figure 7. Yield evolution of motor function compared to the initial moment (I1), according to SMA type, at different moments of the treatment (I5 - before the start of maintenance treatment, I7 - at one year of therapy, I10 – at two years of treatment, and I13 – at three years of treatment)

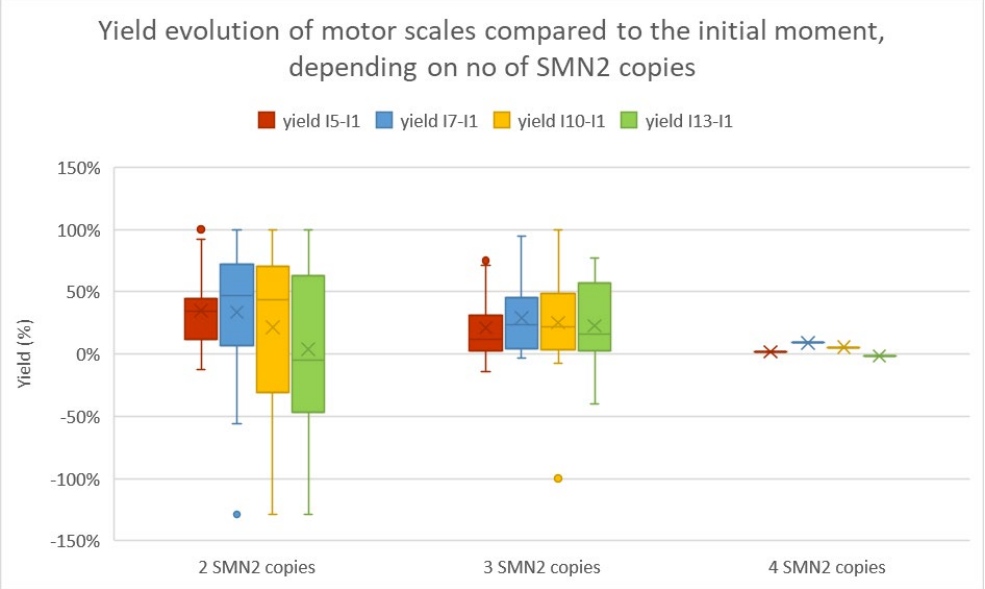

Figure 8. Yield evolution of motor function compared to the initial moment (I1), according to no of SMN2 copies, at different moments of the treatment (I5 - before the start of maintenance treatment, I7 - at one year of therapy, I10 – at two years of treatment, and I13 – at three years of treatment)

3.5. Correlations between parameters

For an objective analysis of the evolution of the obtained results, the percentage values of the pNF-H level in the serum and in the CSF were calculated compared to the initial moment and the yield of the evolution on the motor scales during the three years monitored, with an emphasis on the initial moments, at transition to maintenance therapy (6 months of treatment, at dose number 5) and after 3 years of treatment and a series of statistically significant positive and negative correlations were obtained, with different degrees of correlation.

In SMA type 1, at the initiation of nusinersen treatment, statistically significant correlations were observed primarily with pNF-H neurofilament levels in the CSF. This correlation was weakly negative with the score obtained on the CHOP INTEND scales specific to SMA type 1 (r = -0.426) and with the number of copies of the SMN2 gene (r = -0.429). Conversely, there was a positive and moderate correlation (r = 0.632) with serum pNF-H neurofilament levels.

Furthermore, the serum creatinine level at treatment initiation showed a statistically significant, positive, and very strong correlation (r = 1.000) with CSF and serum pNF-H neurofilament levels three years after treatment initiation.

A statistically significant and very strong correlation (r = 1,000) was obtained between the level of neurofilaments in the CSF at the beginning and after three years of treatment with nusinersen. The values of the CHOP INTEND scales at the start of the maintenance treatment were positively but weakly correlated with the number of SMN2 copies (r = 0.420). Nevertheless, following 3 years of treatment, there was a strong negative correlation (r = -0.850) between the patient's age at treatment initiation and their values, and a very strong correlation (r = 1.000) with the evolution yield over the same period.

The serum values of pNF-H after 3 years of treatment were strongly positively correlated with the percentage value of the evolution of the level of pNF-H in the CSF compared to the value obtained after 6 months of treatment (r = 0.836) and the performance on the motor scale at 6 months of treatment (r = 0.847) and very strongly with the yield after three years (r = 0.957). The percentage of pNF-H neurofilaments in the CSF at the time of starting the maintenance treatment compared to the initial value was weakly positively correlated with the number of SMN2 copies (r = 0.494), but the percentage after three years of treatment compared to the initial value was strongly positively correlated with age at initiation (r= 0.705) and those compared to the start of maintenance treatment with serum creatinine value after 3 years of treatment (r = 0.836).

In SMA type 2, statistically significant, negative, and moderate correlations as power were found between the age of the patients at the beginning of the treatment and the score on the motor scales at the beginning (r = -0.656), serum creatinine and performance at the start of the maintenance treatment (r = -0.596, respectively –0.619), and strongly negatively correlated with the yield after three years of therapy with nusinersen (r = -0.809). The age at initiation correlated positively but weakly with the value of neurofilaments in the CSF after three years of treatment (r = 0.492) and moderately with the percentage level of neurofilaments between the beginning and the end of the loading period with nusinersen (r = 0.526), the percentage with which the score on the motor scale at the beginning was weakly negatively correlated (r = -0.451).

The HFMSE score at the beginning had a moderately positive correlation with the yield from the beginning of the maintenance treatment (r = 0.573) and after three years of treatment (r = 0.601). The level of neurofilaments in the serum at the start of treatment was very strongly positively correlated both with the level of neurofilaments at the beginning of the maintenance period (r = 0.980) as well as with that after three years of treatment (r = 1.000) and with the yield at the beginning of the maintenance period maintenance (r = 0.955). The value of neurofilaments in the CSF at the beginning correlated positively and strongly with the value of neurofilaments in the serum (r = 0.891) and moderately with the yield (r = 0.573) at the beginning of the maintenance period and with the yield after three years of treatment (r = 0.601). Serum creatinine at the beginning was moderately positively correlated with the level of neurofilaments in the CSF at the beginning of the maintenance period (r= 0.685) and with their percentage value compared to the initial value (r = 0.582).

The level of pNF-H neurofilaments in the serum at the beginning of the maintenance period had a strong negative correlation with the percentage of neurofilaments in the CSF after three years of treatment relative to the initial value (r = -0.900). The level of pNF-H in the CSF at the beginning of the maintenance period correlated very strongly and positively with the percentage of pNF-H in the serum after three years of treatment relative to the initial value (r = 0.971).

Serum creatinine at the beginning of the maintenance period had a weak positive correlation with the performance in the same period (r = 0.490), a moderate and negative correlation with age at initiation (r = -0.596), and a very strong and negative correlation with the percentage value of neurofilaments in the serum compared to the initial moment (r = -0.974).

The HFMSE score at the beginning of the maintenance period correlated strongly negatively with age at initiation (r = -0.731), strongly positively with the performance of the same period (r = 0.809), and with that of three years of treatment (r = 0.759), weakly negatively with the evolution of pNF-H in CSF between initiation and the beginning of maintenance (r = -0.453) and very strongly negative with the percentage of pNF-H in serum after three years of treatment compared to the level at the time of initiation (r = -0.919).

After three years of treatment, the value of neurofilaments in the CSF correlated weakly positively with age at the initiation of therapy (r = 0.492) and moderately positively with the percentage evolution of the pNF-H level in the CSF between the time of the start of the maintenance treatment and that of the initiation of the medication (r = 0.620), an aspect also observed for the evolution of pNF-H in the serum compared to the percentage value of the evolution of pNF-H in the serum between the start of the maintenance treatment compared to the initial value, with a very strongly negative correlation (r = -1.000), and serum creatinine was very strongly positively correlated both with the value of neurofilaments in the serum after three years of treatment (r = 1.000), as well as with their percentage value relative to the level at the start of the maintenance treatment (r = 0.984).

The HFMSE score after three years of treatment had a strong and negative correlation with age at initiation (r = -0.756), moderately and negatively with the percentage evolution of pNF-H in the CSF during the loading period (r = -0.537), and moderately and positively with the yield at the beginning of the maintenance period (r = 0.660).

We observed a very strong positive correlation (r = 0.982) between the percentage change in serum neurofilament levels after three years of treatment compared to the initial values and similarly strong correlations between these percentage values and those at the beginning of the maintenance period (r = 1.000). Additionally, there was a very strong positive correlation (r = 0.984) between both percentage values and serum creatinine levels after three years of treatment.

The percentage change in pNF-H levels in the CSF during the drug loading period showed a moderate positive correlation with the percentage change during the three years of treatment (r = 0.512) and with the pNF-H levels after three years of treatment (r = 0.620), but weakly negative correlation with the overall outcome during these three years (r = -0.492). There was a strong positive correlation between the overall outcome during these three years and the outcome at the beginning of the maintenance period (r = 0.746).

In SMA type 3, the age of the patients at the start of the treatment correlated moderately negatively with the percentage evolution of pNF-H in the CSF for the same period (r = -0.603). The number of copies of the SMN2 gene was moderately negatively correlated with the creatinine level at the beginning of the maintenance treatment (r = -0.603), while the creatinine level at the start of the therapy was strongly positively correlated with the performance at the beginning of the maintenance period (r = 0.779), moderately negative with the pNF-H level in the CSF at the beginning of the maintenance period (r = -0.638) and very strongly negative with the percentage evolution of the pNF-H level in the CSF during the maintenance period (r = -1.000).

The level of pNF-H in the CSF at the start of treatment correlated moderately positively with the level of creatinine and strongly positively with neurofilaments in the serum after three years of treatment (r= 0.656, respectively r = 0.748) and very strongly positively with the percentage evolution of the level of pNF-H in the CSF between the level after three years of treatment compared to that at the beginning of the maintenance period (r = 1.000).

The HFMSE score at initiation exhibited a moderate negative correlation with the number of copies of the SMN2 gene (r = -0.636), a moderate positive correlation with serum creatinine at initiation (r = 0.643) and after three years of treatment (r = 0.679), and a strong positive correlation with the outcomes at the beginning of the maintenance period (r = 0.792) and after three years of treatment (r = 0.800).

Serum creatinine at the beginning of the maintenance treatment correlated strongly positively with the yield at the same time (r = 0.732), moderately positively with that after three years of treatment (r = 0.660), and moderately negatively with the level of neurofilaments in the CSF at the beginning of the maintenance period (r = -0.580).

The HFMSE score at the beginning of the maintenance period displayed a strong positive correlation with performance during the same period (r = 0.856) and after three years of treatment (r = 0.867). It also showed a moderate positive correlation with serum creatinine levels after three years of treatment (r = 0.685), which in turn correlated moderately positively with the outcome after three years of treatment (r = 0.626) and strongly positively with the outcome at the beginning of the maintenance period (r = 0.783). Additionally, it correlated moderately positively with the percentage change in pNF-H levels in the CSF compared to the initial value for the value after three years of treatment (r = 0.642) and strongly positively for the value from the beginning of the maintenance treatment (r = 0.711).

The HFMSE score after three years of treatment exhibited a moderate positive correlation with serum creatinine levels after the same duration (r = 0.613), with the percentage change in pNF-H levels during the maintenance period (r = 0.650) and a strong positive correlation with the outcome at the beginning of the maintenance period (r = 0.868). Furthermore, it showed a very strong positive correlation with the outcome after three years of treatment (r = 0.933) and a very strong positive correlation with the outcome during the loading treatment period (r = 0.920).

Several correlations emerged when analyzing the results based on the number of copies of the SMN2 gene within the group possessing two copies of SMN2. There was a strong positive correlation between the age at treatment initiation and the type of SMA (r

= 0.756), a moderate positive correlation with serum creatinine levels at initiation (r = 0.644), and with the percentage change in pNF-H levels in the CSF during treatment (r = 0.574). Weak negative correlations were observed with the initial pNF-H levels in the CSF (r = -0.421), and weak positive correlations were noted with the percentage change in pNF-H levels during the loading period (r = 0.467) and with the initial pNF-H levels in the CSF (r = -0.437). Furthermore, there was a moderate positive correlation with serum creatinine levels after three years of treatment (r = 0.584), and the percentage change in pNF-H levels in the CSF correlated with the type of SMA after three years (r = 0.531). It showed a strong positive correlation with the HFMSE score after three years (r = 0.847).

Serum creatinine levels at the beginning strongly positively correlated with pNF-H levels in the CSF after three years of treatment (r = 0.855), and pNF-H levels in the CSF at the beginning were very strongly positively correlated with those in the serum after three years of treatment (r = 0.940). The CHOP INTEND score at initiation moderately positively correlated with the outcome during the loading period (r = 0.614), while the HFMSE score at initiation strongly positively correlated with the outcome during the loading period (r = 0.788) and very strongly positively with the outcome at the end of the study (r = 0.967).

The HFMSE score at the beginning of the maintenance period showed strong positive correlations with the serum creatinine level at that time (r = 0.775), the overall outcome (r = 0.835), and the percentage change in pNF-H levels in the CSF during the study (r = 0.831). It also exhibited very strong positive correlations with the type of SMA (r = 0.922) and the outcome after three years of treatment (r = 0.974).

Following three years of treatment, the pNF-H level in the CSF strongly positively correlated with that in the serum (r = 0.751), and the HFMSE score showed strong positive correlations with the type of SMA (r = 0.890), the serum creatinine level (r = 0.858), and the outcome from the beginning of the loading period (r = 0.888), and very strong positive correlation with the overall outcome (r = 0.985).

The percentage change in pNF-H levels during the loading period weakly correlated with the age at initiation (r = 0.467), while the percentage change in pNF-H levels in the CSF during the three years of treatment moderately positively correlated with the age at initiation (r = 0.574) and the type of SMA (r = 0.531), and strongly positively with the percentage change in pNF-H levels during the loading period (r = 0.810).

The yield of the HFMSE score at the beginning of the maintenance period was strongly positively correlated with the creatinine value at that time (r = 0.882). The yield after three years of treatment was moderately positively correlated with the type of SMA (r = 0.847) and serum creatinine at the beginning of the maintenance period (r = 0.844) and very strongly positive with the yield after the loading period (0.931). In the group with three copies of the SMN2 gene, the age at the onset of treatment showed moderate positive correlations with the type of SMA (r = 0.547) and with the variation of pNF-H levels during the maintenance period (r = 0.565). Moreover, it exhibited a strong positive correlation with the percentage change in pNF-H levels in serum over the entire treatment period (r = 0.883). Age at initiation was moderately negatively correlated with serum creatinine at the beginning of the maintenance period (r = -0.575) and with the performance on the HFMSE scale after three years of treatment (r = -0.529).

For the type of SMA, the correlations were moderately positive with the percentage evolution of the pNF-H level in the serum during the entire treatment period (r = 0.639) and for the maintenance treatment period (r = 0.598) and weakly negative with the percentage evolution of pNF- H from CSF during the loading period (r = -0.380). The initial level of pNF-H in the serum correlated moderately positively with the initial level in the CSF (r = 0.669) and that in the serum from the beginning of the maintenance period (r = 0.639) and with the percentage of the evolution of pNF-H in the CSF during the maintenance period (r = 0.613) and very strongly positive with the HFMSE yield for the loading period (r = 0.952).

The HFMSE score at the beginning exhibited weak positive correlations with the type of SMA (r = 0.458) and the serum creatinine level at the beginning (r = 0.416) and after three years of treatment (r = 0.469), but moderately positive correlations with the outcome at the start of the maintenance treatment period (r = 0.619) and after three years of

treatment (r = 0.541). The creatinine value at the beginning of the maintenance period weakly positively correlated with the outcome at that moment (r = 0.474) and after three years of treatment (r = 0.462). Furthermore, the pNF-H level in the CSF at the beginning of the maintenance period showed a very strong positive correlation with the percentage change in pNF-H levels in the serum during the loading period (r = 0.980).

At the start of the maintenance period, the CHOP INTEND and HFMSE scores exhibited a strongly positive correlation with the outcome (r = 0.954, respectively, r = 0.800). The HFMSE score at the beginning of the maintenance period showed weak negative correlations with the age at initiation (r = -0.429) and the percentage change in pNF-H levels in the CSF during the initiation period (r = -0.417). After three years of treatment, there was a slight positive correlation (r = 0.414) with the serum creatinine level and a strong positive correlation with the initial level of pNF-H in the serum (r = 0.798), the outcome at the beginning of the maintenance period (r = 0.800), and a moderate positive correlation after three years of treatment (r = 0.689).

The serum creatinine level after three years of treatment displayed a very strong positive correlation with the percentage change in pNF-H levels in the serum during the maintenance period (r = 0.949) but only weakly positive correlations with the percentage change in pNF-H levels in the CSF during treatment (r = 0.439) and the HFMSE outcome after three years of treatment (r = 0.491).

The HFMSE score after three years of treatment exhibited a moderate negative correlation with age at initiation (r = -0.537) and weak negative correlations with the percentage change in pNF-H levels in CSF during the loading period (r = -0.437). It showed a strong positive correlation with the outcome of the loading period (r = 0.739) and after three years of treatment and a moderate positive correlation with the serum creatinine level (r = 0.862 and r = 0.560, respectively).

The percentage change in pNF-H levels in the serum during the loading period was very strongly positively correlated with the percentage change in pNF-H levels in the CSF during the same period (r = 0.974) and with the level of pNF-H in the CSF at the beginning of the maintenance period (r = 0.980).

The percentage change in pNF-H levels in the serum during treatment was strongly positively correlated with age at initiation (r = 0.883) and very strongly positively correlated with the percentage change in pNF-H levels in the serum during the maintenance period (r = 0.929). It was also very strongly negatively correlated with the level of pNF-H in CSF after three years of treatment (r = -0.944) and moderately positively correlated with the type of SMA (r = 0.639).

The percentage change in pNF-H levels in the serum during the maintenance period was moderately positively correlated with age at initiation (r = 0.565) and the type of SMA (r = 0.598) and very strongly positively correlated with the serum creatinine level after three years of treatment (r = 0.949).

The percentage change in pNF-H levels in CSF during the loading period was weakly negatively correlated with the type of SMA (r = -0.380), moderately positively correlated with that during the entire treatment (r = 0.656), and with the value of pNF-H in the serum at initiation (r = 0.613).

The HFMSE outcome during the loading period showed a very strong positive correlation with the pNF-H level at initiation (r = 0.952), a weakly positive correlation with the serum creatinine level at the beginning of the loading period (r = 0.474), and a strongly positive correlation with the outcome after three years of treatment (r = 0.823).

The HFMSE outcome after three years of treatment correlated moderately negatively with age at initiation (r = -0.529) and weakly positively with serum creatinine at the beginning of the maintenance period (r = 0.462).

4. Discussion

After analyzing the results obtained for the 69 patients included in this study, we observed favorable outcomes in all patients from both clinical and paraclinical perspectives. Notably, patients who received treatment earlier showed better results, a

finding that aligns with existing evidence in the specialized literature [46]. Clinically, scores on motor evaluation scales improved. Paraclinically, favorable changes were observed in the levels of investigated parameters, such as serum creatinine (indicative of muscle atrophy) and pNF-H levels in cerebrospinal fluid (CSF) and serum (reflecting motor neuron damage).

The highest pNF-H levels in CSF were observed before the initiation of nusinersen treatment across all time points analyzed (before initiation - I1, at the beginning of maintenance treatment - I5, after one year - I7, and after two and three years - I10 and I13, respectively). This trend was consistent across different types of SMA and for patients with varying copies of the SMN2 gene [44,47–51]. Notably, patients with SMA type 1 and those with 2 copies of the SMN2 gene exhibited the highest pNF-H levels, aligning with findings in the literature regarding disease severity factors [47,48].

The most significant decrease in CSF pNF-H levels occurred between treatment initiation and the beginning of maintenance therapy, regardless of SMA type or SMN2 gene copy number. During the maintenance period, CSF pNF-H levels generally remained lower than those at the beginning, except for two patients with SMA type 1 and 2 copies of the SMN2 gene before the 7th and 10th doses of the drug, whose subsequent trends aligned with their respective patient groups.

A gradual decrease in CSF pNF-H levels relative to baseline was observed, particularly at the start of maintenance treatment. Variations were based on the number of SMN2 gene copies and SMA type.

The serum level of pNF-H also had the highest values at treatment initiation for patients with 2 copies of the SMN2 gene compared to those with 3 or 4 copies. Still, the group of patients with SMA type 2 recorded the highest values of this parameter, while those with SMA type 1 and 3 had similar values.

Serum creatinine levels, indicative of muscle atrophy, decreased during the first 6 months of nusinersen treatment but increased during maintenance treatment, especially in SMA type 2 and 3 patients. Patients diagnosed with SMA type 1 exhibited relatively minor fluctuations, although they continued to increase after three years of treatment, aligning with the atrophy patterns observed in earlier research [25,52,53]. Furthermore, patients showed increases in serum creatinine levels after 3 years of nusinersen treatment compared to baseline, notably correlated with decreased muscle loss and increased muscle metabolism, consistent with findings in existing literature [26,54].

Limitations

The methods employed to evaluate patient response to nusinersen treatment for spinal muscular atrophy (SMA) are constrained by the inherent limitations of each biomarker analyzed. Moreover, the study encounters obstacles due to the small size of the patient cohort, given the rarity of SMA as a disease.

Motor performance evaluation relies on patient cooperation, which can be challenging, especially in pediatric populations with fluctuating moods and health statuses. Furthermore, integrating treatment with adjunct therapies like physical therapy introduces additional complexity. Physical therapy is pivotal in improving various aspects of motor function, including posture, muscle tone and strength, and range of motion [55]. These improvements translate into enhanced quality of life for patients and their families, underscoring the importance of a holistic approach to SMA management.

Paraclinical parameters, while less influenced by external factors or patient cooperation, are not specific to the type of disease and are influenced by individual metabolism.

In addition, the study did not include intensely hemolyzed CSF samples or hemolytic, lipemic, or icteric serum samples.

In the serum samples analyzed for the serum creatinine level, there were no hemolyzed samples.

Future Work Directions

To better understand the correlation strength between analyzed parameters and patient evolution, longer-term studies involving larger patient cohorts and different disease-modifying drugs are necessary. These longer-term studies would enable the development of optimal treatment protocols tailored to each patient's genotypic and phenotypic characteristics.

In the future, the correlation of paraclinical data with electrophysiological parameters in the evolution under treatment of patients with SMA could bring new data related to the evolution and prognosis of patients with different forms of the disease under the influence of specific treatments.

5. Conclusions

SMA presents as a debilitating condition with a severe prognosis, often culminating in fatality, particularly in its severe forms. Each patient, and each group of patients, exhibits unique characteristics and responses to treatment. Hence, a thorough assessment of individual performance necessitates consideration of multiple facets, encompassing clinical consultation to evaluate overall health status and motor function, along with paraclinical evaluation focusing on the progression of biomarkers like pNF-H levels in serum and CSF, as well as serum creatinine. Each parameter offers unique insights into disease advancement and treatment effectiveness.

Our study reveals the fact that the highest values of pNF-H as a marker of neuronal degradation were obtained in the CSF of patients with the most severe forms of the disease (SMA type 1), and after treatment with nusinersen, the level of pNF-H decreased as a response to the treatment after the loading period both in absolute value and relative to the initial value up to a level probably due to the normal metabolism of the motor neurons. It also observed a decrease in the level of pNF-H in the serum of the treated patients and an increase in serum creatinine level due to increased muscle activity during the 3 years of treatment. Most of the patients obtained increases in scores on the functional motor scales. However, the best results were also obtained for patients with SMA type 1, probably due to the early initiation of treatment during the disease until the regression of motor acquisitions and neuromuscular impairment. For patients with SMA type 2 and 3, the evolution of biomarkers and motor performance was favorable but with more modest results.

The results of the study, combined with those from the literature, support the improvement of functional and paraclinical parameters as a result of the administration of a disease-modifying treatment and the possibility of verifying the effectiveness of the therapy for decreasing neuronal degeneration as a result of the SMN protein deficiency - by following the evolution of the pNF-H level in the CSF, and in the serum.

The studied biomarkers are correlated to different degrees during the treatment, depending on the type of SMA, the number of copies of the SMN2 gene, the time elapsed since the onset of symptoms and their severity, the degree of cooperation of the patients, and compliance for physical therapy.

The pediatric population with SMA should be monitored by all available means to obtain a complete picture of the evolution and prognosis of the patients under treatment and to adapt the adjuvant therapy according to the needs.

The treatment of SMA is complex, requiring a multidisciplinary approach to maximize the results of disease-modifying therapy that addresses the leading cause of the patient's motor status impairment by increasing the amount of available SMN protein, thus preventing neuron degradation from this cause.

Increasing the patient's quality of life and obtaining functional independence for each stage of the evolution of the disease under treatment is also based on individual exercise and depends on the patient's will, requiring a combination of physical and occupational therapy.

Monitoring the level of pNF-H neurofilaments provides information about the evolution of motor neuron degeneration, and serum creatinine, together with the assessment of specific motor scales, reflects the degree of muscle activity.

The decrease in the level of neurofilaments in the serum and CSF demonstrates the slowing down or even stopping of the abnormal neuronal degradation. The increase in serum creatinine is due to the intensification of involuntary (for example, breathing or swallowing) and voluntary muscle activity (assessed by the scores obtained on the functional motor scales) due to the improvement of neuronal functionality.

The unfavorable evolution of these parameters (increase in pNF-H level and decrease in creatinine level or functional scores) translates into a lack of response to the chosen treatment strategy.

A favorable evolution of the level of neurofilaments, but a stagnation or a decrease in the level of creatinine and the results obtained in the functional evaluations may suggest an effective drug treatment, but a lack of compliance of the patient to the adjuvant therapy or the need to intensify it by increasing the number of sessions of physical therapy or their duration or the complexity of the exercises performed.

Swift diagnosis and prompt initiation of specific disease-modifying treatments are crucial. Eary initiating treatment prolongs life expectancy and increases the likelihood of better motor function recovery. Therefore, advocating for and establishing neonatal screening programs worldwide is crucial. Early detection can profoundly impact the course of SMA, providing patients with the opportunity for more favorable outcomes and an enhanced quality of life.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written consent has been obtained from the patient(s) to publish this paper.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Shpargel, K.B.; Matera, A.G. Gemin Proteins Are Required for Efficient Assembly of Sm-Class Ribonucleoproteins. Proc. Natl. Acad. Sci. 2005, 102, 17372–17377, doi:10.1073/pnas.0508947102.
2. Frugier, T.; Nicole, S.; Cifuentes-Diaz, C.; Melki, J. The Molecular Bases of Spinal Muscular Atrophy. Curr. Opin. Genet. Dev. 2002, 12, 294–298, doi:10.1016/S0959-437X(02)00301-5.
3. Lorson, C.L.; Hahnen, E.; Androphy, E.J.; Wirth, B. A Single Nucleotide in the SMN Gene Regulates Splicing and Is Responsible for Spinal Muscular Atrophy. Proc. Natl. Acad. Sci. 1999, 96, 6307–6311, doi:10.1073/pnas.96.11.6307.

4. Sivaramakrishnan, M.; McCarthy, K.D.; Campagne, S.; Huber, S.; Meier, S.; Augustin, A.; Heckel, T.; Meistermann, H.; Hug, M.N.; Birrer, P.; et al. Binding to SMN2 Pre-MRNA-Protein Complex Elicits Specificity for Small Molecule Splicing Modifiers. Nat. Commun. 2017, 8, 1476, doi:10.1038/s41467-017-01559-4.
5. Harada, Y.; Sutomo, R.; Sadewa, A.H.; Akutsu, T.; Takeshima, Y.; Wada, H.; Matsuo, M.; Nishio, H. Correlation between SMN2 Copy Number and Clinical Phenotype of Spinal Muscular Atrophy: Three SMN2 Copies Fail to Rescue Some Patients from the Disease Severity. J. Neurol. 2002, 249, 1211–1219, doi:10.1007/s00415-002-0811-4.
6. Gidaro, T.; Servais, L. Nusinersen Treatment of Spinal Muscular Atrophy: Current Knowledge and Existing Gaps. Dev. Med. Child Neurol. 2019, 61, 19–24, doi:10.1111/dmcn.14027.
7. Axente, M.; Mirea, A.; Sporea, C.; Pădure, L.; Drăgoi, C.M.; Nicolae, A.C.; Ion, D.A. Clinical and Electrophysiological Changes in Pediatric Spinal Muscular Atrophy after 2 Years of Nusinersen Treatment. Pharmaceutics 2022, 14, 2074, doi:10.3390/pharmaceutics14102074.
8. Pierzchlewicz, K.; Kępa, I.; Podogrodzki, J.; Kotulska, K. Spinal Muscular Atrophy: The Use of Functional Motor Scales in the Era of Disease-Modifying Treatment. Child Neurol. Open 2021, 8, 2329048X2110087, doi:10.1177/2329048X211008725.
9. Arnold, W.D.; Kassar, D.; Kissel, J.T. Spinal Muscular Atrophy: Diagnosis and Management in a New Therapeutic Era. Muscle Nerve 2015, 51, 157–167, doi:10.1002/mus.24497.
10. Rudnik-Schöneborn, S.; Berg, C.; Zerres, K.; Betzler, C.; Grimm, T.; Eggermann, T.; Eggermann, K.; Wirth, R.; Wirth, B.; Heller, R. Genotype–Phenotype Studies in Infantile Spinal Muscular Atrophy (SMA) Type I in Germany: Implications for Clinical Trials and Genetic Counselling. Clin. Genet. 2009, 76, 168–178, doi:10.1111/j.1399-0004.2009.01200.x.
11. Finkel, R.; Bertini, E.; Muntoni, F.; Mercuri, E. 209th ENMC International Workshop: Outcome Measures and Clinical Trial Readiness in Spinal Muscular Atrophy 7–9 November 2014, Heemskerk, The Netherlands. Neuromuscul. Disord. 2015, 25, 593–602, doi:10.1016/j.nmd.2015.04.009.
12. Verhaart, I.E.C.; Robertson, A.; Wilson, I.J.; Aartsma-Rus, A.; Cameron, S.; Jones, C.C.; Cook, S.F.; Lochmüller, H. Prevalence, Incidence and Carrier Frequency of 5q–Linked Spinal Muscular Atrophy – a Literature Review. Orphanet J. Rare Dis. 2017, 12, 124, doi:10.1186/s13023-017-0671-8.
13. Lally, C.; Jones, C.; Farwell, W.; Reyna, S.P.; Cook, S.F.; Flanders, W.D. Indirect Estimation of the Prevalence of Spinal Muscular Atrophy Type I, II, and III in the United States. Orphanet J. Rare Dis. 2017, 12, 175, doi:10.1186/s13023-017-0724-z.
14. Scarciolla, O.; Stuppia, L.; De Angelis, M.V.; Murru, S.; Palka, C.; Giuliani, R.; Pace, M.; Di Muzio, A.; Torrente, I.; Morella, A.; et al. Spinal Muscular Atrophy Genotyping by Gene Dosage Using Multiple Ligation-Dependent Probe Amplification. Neurogenetics 2006, 7, 269–276, doi:10.1007/s10048-006-0051-3.
15. Mercuri, E.; Sumner, C.J.; Muntoni, F.; Darras, B.T.; Finkel, R.S. Spinal Muscular Atrophy. Nat. Rev. Dis. Prim. 2022, 8, 52, doi:10.1038/s41572-022-00380-8.
16. Monnette, A.; Chen, E.; Hong, D.; Bazzano, A.; Dixon, S.; Arnold, W.D.; Shi, L. Treatment Preference among Patients with Spinal Muscular Atrophy (SMA): A Discrete Choice Experiment. Orphanet J. Rare Dis. 2021, 16, 36, doi:10.1186/s13023-020-01667-3.
17. Mirea, A.; Shelby, E.-S.; Axente, M.; Badina, M.; Padure, L.; Leanca, M.; Dima, V.; Sporea, C. Combination Therapy with Nusinersen and Onasemnogene Abeparvovec-Xioi in Spinal Muscular Atrophy Type I. J. Clin. Med. 2021, 10, 5540, doi:10.3390/jcm10235540.
18. Meneri, M.; Abati, E.; Gagliardi, D.; Faravelli, I.; Parente, V.; Ratti, A.; Verde, F.; Ticozzi, N.; Comi, G.P.; Ottoboni, L.; et al. Identification of Novel Biomarkers of Spinal Muscular Atrophy and Therapeutic Response by Proteomic and Metabolomic Profiling of Human Biological Fluid Samples. Biomedicines 2023, 11, 1254, doi:10.3390/biomedicines11051254.

19. Finkel, R.S.; Mercuri, E.; Darras, B.T.; Connolly, A.M.; Kuntz, N.L.; Kirschner, J.; Chiriboga, C.A.; Saito, K.; Servais, L.; Tizzano, E.; et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. N. Engl. J. Med. 2017, 377, 1723–1732, doi:10.1056/NEJMoa1702752.
20. Colella, P.; Ronzitti, G.; Mingozzi, F. Emerging Issues in AAV-Mediated In Vivo Gene Therapy. Mol. Ther. - Methods Clin. Dev. 2018, 8, 87–104, doi:10.1016/j.omtm.2017.11.007.
21. Darras, B.T.; Masson, R.; Mazurkiewicz-Bełdzińska, M.; Rose, K.; Xiong, H.; Zanoteli, E.; Baranello, G.; Bruno, C.; Vlodavets, D.; Wang, Y.; et al. Risdiplam-Treated Infants with Type 1 Spinal Muscular Atrophy versus Historical Controls. N. Engl. J. Med. 2021, 385, 427–435, doi:10.1056/NEJMoa2102047.
22. Ribault, S.; Rippert, P.; Jain, M.; Le Goff, L.; Genod, D.V.; Barriere, A.; Berruyer, A.; Garde, C.; Tinat, M.; Pons, C.; et al. Psychometric Characteristics of the Motor Function Measure in Neuromuscular Diseases: A Systematic Review1. J. Neuromuscul. Dis. 2023, 10, 301–314, doi:10.3233/JND-230001.
23. Ramsey, D.; Scoto, M.; Mayhew, A.; Main, M.; Mazzone, E.S.; Montes, J.; de Sanctis, R.; Dunaway Young, S.; Salazar, R.; Glanzman, A.M.; et al. Revised Hammersmith Scale for Spinal Muscular Atrophy: A SMA Specific Clinical Outcome Assessment Tool. PLoS One 2017, 12, e0172346, doi:10.1371/journal.pone.0172346.
24. Glanzman, A.M.; Mazzone, E.; Main, M.; Pelliccioni, M.; Wood, J.; Swoboda, K.J.; Scott, C.; Pane, M.; Messina, S.; Bertini, E.; et al. The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND): Test Development and Reliability. Neuromuscul. Disord. 2010, 20, 155–161, doi:10.1016/j.nmd.2009.11.014.
25. Alves, C.R.R.; Zhang, R.; Johnstone, A.J.; Garner, R.; Nwe, P.H.; Siranosian, J.J.; Swoboda, K.J. Serum Creatinine Is a Biomarker of Progressive Denervation in Spinal Muscular Atrophy. Neurology 2020, 94, e921–e931, doi:10.1212/WNL.0000000000008762.
26. Freigang, M.; Wurster, C.D.; Hagenacker, T.; Stolte, B.; Weiler, M.; Kamm, C.; Schreiber-Katz, O.; Osmanovic, A.; Petri, S.; Kowski, A.; et al. Serum Creatine Kinase and Creatinine in Adult Spinal Muscular Atrophy under Nusinersen Treatment. Ann. Clin. Transl. Neurol. 2021, 8, 1049–1063, doi:10.1002/acn3.51340.
27. Blasi, L.; Sabbatini, D.; Fortuna, A.; Querin, G.; Martinelli, I.; Vianello, S.; Bertolin, C.; Pareyson, D.; Pennuto, M.; Pegoraro, E.; et al. The Value of Serum Creatinine as Biomarker of Disease Progression in Spinal and Bulbar Muscular Atrophy (SBMA). Sci. Rep. 2023, 13, 17311, doi:10.1038/s41598-023-44419-6.
28. Wang, L.; Chen, M.; He, R.; Sun, Y.; Yang, J.; Xiao, L.; Cao, J.; Zhang, H.; Zhang, C. Serum Creatinine Distinguishes Duchenne Muscular Dystrophy from Becker Muscular Dystrophy in Patients Aged ≤3 Years: A Retrospective Study. Front. Neurol. 2017, 8, 196, doi:10.3389/fneur.2017.00196.
29. Yuan, A.; Nixon, R.A. Neurofilament Proteins as Biomarkers to Monitor Neurological Diseases and the Efficacy of Therapies. Front. Neurosci. 2021, 15, 689938, doi:10.3389/fnins.2021.689938.
30. Yuan, A.; Rao, M. V.; Veeranna; Nixon, R.A. Neurofilaments and Neurofilament Proteins in Health and Disease. Cold Spring Harb. Perspect. Biol. 2017, 9, a018309, doi:10.1101/cshperspect.a018309.
31. Hoffman, P.N.; Lasek, R.J. The Slow Component of Axonal Transport. Identification of Major Structural Polypeptides of the Axon and Their Generality among Mammalian Neurons. J. Cell Biol. 1975, 66, 351–366, doi:10.1083/jcb.66.2.351.
32. Yuan, A.; Rao, M. V.; Sasaki, T.; Chen, Y.; Kumar, A.; Veeranna; Liem, R.K.H.; Eyer, J.; Peterson, A.C.; Julien, J.-P.; et al. α-Internexin Is Structurally and Functionally Associated with the Neurofilament Triplet Proteins in the Mature CNS. J. Neurosci. 2006, 26, 10006–10019, doi:10.1523/JNEUROSCI.2580-06.2006.
33. Yuan, A.; Sasaki, T.; Kumar, A.; Peterhoff, C.M.; Rao, M. V.; Liem, R.K.; Julien, J.-P.; Nixon, R.A. Peripherin Is a Subunit of Peripheral Nerve Neurofilaments: Implications for

Differential Vulnerability of CNS and Peripheral Nervous System Axons. J. Neurosci. 2012, 32, 8501–8508, doi:10.1523/JNEUROSCI.1081-12.2012.
34. Khalil, M.; Teunissen, C.E.; Otto, M.; Piehl, F.; Sormani, M.P.; Gattringer, T.; Barro, C.; Kappos, L.; Comabella, M.; Fazekas, F.; et al. Neurofilaments as Biomarkers in Neurological Disorders. Nat. Rev. Neurol. 2018, 14, 577–589, doi:10.1038/s41582-018-0058-z.
35. Heckler, I.; Venkataraman, I. Phosphorylated Neurofilament Heavy Chain: A Potential Diagnostic Biomarker in Amyotrophic Lateral Sclerosis. J. Neurophysiol. 2022, 127, 737–745, doi:10.1152/jn.00398.2021.
36. Yuan, A.; Rao, M. V.; Veeranna; Nixon, R.A. Neurofilaments at a Glance. J. Cell Sci. 2012, 125, 3257–3263, doi:10.1242/jcs.104729.
37. Petzold, A. Neurofilament Phosphoforms: Surrogate Markers for Axonal Injury, Degeneration and Loss. J. Neurol. Sci. 2005, 233, 183–198, doi:10.1016/j.jns.2005.03.015.
38. Wang, H.; Wu, M.; Zhan, C.; Ma, E.; Yang, M.; Yang, X.; Li, Y. Neurofilament Proteins in Axonal Regeneration and Neurodegenerative Diseases. Neural Regen. Res. 2012, 7, 620, doi:10.3969/j.issn.1673-5374.2012.08.010.
39. Pijnenburg, Y.A.L.; Janssen, J.C.; Schoonenboom, N.S.M.; Petzold, A.; Mulder, C.; Stigbrand, T.; Norgren, N.; Heijst, H.; Hack, C.E.; Scheltens, P.; et al. CSF Neurofilaments in Frontotemporal Dementia Compared with Early Onset Alzheimer's Disease and Controls. Dement. Geriatr. Cogn. Disord. 2007, 23, 225–230, doi:10.1159/000099473.
40. Rosengren, L.E.; Karlsson, J.; Karlsson, J.; Persson, L.I.; Wikkelsø, C. Patients with Amyotrophic Lateral Sclerosis and Other Neurodegenerative Diseases Have Increased Levels of Neurofilament Protein in CSF. J. Neurochem. 1996, 67, 2013–2018, doi:10.1046/j.1471-4159.1996.67052013.x.
41. Brkušanin, M.; Kosać, A.; Branković-Srećković, V.; Jovanović, K.; Perić, S.; Karanović, J.; Matijašević Joković, S.; Garai, N.; Pešović, J.; Nikolić, D.; et al. Phosphorylated Neurofilament Heavy Chain in Cerebrospinal Fluid and Plasma as a Nusinersen Treatment Response Marker in Childhood-Onset SMA Individuals from Serbia. Front. Neurol. 2024, 15, 1394001, doi:10.3389/fneur.2024.1394001.
42. Andrés-Benito, P.; Vázquez-Costa, J.F.; Ñungo Garzón, N.C.; Colomina, M.J.; Marco, C.; González, L.; Terrafeta, C.; Domínguez, R.; Ferrer, I.; Povedano, M. Neurodegeneration Biomarkers in Adult Spinal Muscular Atrophy (SMA) Patients Treated with Nusinersen. Int. J. Mol. Sci. 2024, 25, 3810, doi:10.3390/ijms25073810.
43. Darras, B.T.; Crawford, T.O.; Finkel, R.S.; Mercuri, E.; De Vivo, D.C.; Oskoui, M.; Tizzano, E.F.; Ryan, M.M.; Muntoni, F.; Zhao, G.; et al. Neurofilament as a Potential Biomarker for Spinal Muscular Atrophy. Ann. Clin. Transl. Neurol. 2019, 6, 932–944, doi:10.1002/acn3.779.
44. De Wel, B.; De Schaepdryver, M.; Poesen, K.; Claeys, K.G. Biochemical and Clinical Biomarkers in Adult <scp>SMA</Scp> 3–4 Patients Treated with Nusinersen for 22 Months. Ann. Clin. Transl. Neurol. 2022, 9, 1241–1251, doi:10.1002/acn3.51625.
45. Rich, K.A.; Fox, A.; Yalvac, M.; Heintzman, S.; Tellez, M.; Bartlett, A.; Severyn, S.; Linsenmayer, M.; Kelly, K.; Reynolds, J.; et al. Neurofilament Levels in CSF and Serum in an Adult SMA Cohort Treated with Nusinersen. J. Neuromuscul. Dis. 2022, 9, 111–119, doi:10.3233/JND-210735.
46. Axente, M.; Sporea, C.; Mirea, A.; Burcea, C.-C.; Ion, D.A. Time-Efficacy in SMA Type 1 and 2 Cases Treated with Nusinersen. Balneo PRM Res. J. 2023, 14, 566, doi:10.12680/balneo.2022.566.
47. Lapp, H.S.; Freigang, M.; Hagenacker, T.; Weiler, M.; Wurster, C.D.; Günther, R. Biomarkers in 5q-Associated Spinal Muscular Atrophy—a Narrative Review. J. Neurol. 2023, 270, 4157–4178, doi:10.1007/s00415-023-11787-y.
48. Badina, M.; Bejan, G.C.; Sporea, C.; Padure, L.; Mirea, A.; Leanca, M.-C.; Axente, M.; Grigoras, F.P.; Bejan, M.; Shelby, E.-S.; et al. Changes in PNFH Levels in Cerebrospinal Fluid and Motor Evolution after the Loading Dose with Nusinersen in Different Types of

Spinal Muscular Atrophy. Medicina (B. Aires). 2023, 59, 1244, doi:10.3390/medicina59071244.
49. Wurster, C.D.; Günther, R.; Steinacker, P.; Dreyhaupt, J.; Wollinsky, K.; Uzelac, Z.; Witzel, S.; Kocak, T.; Winter, B.; Koch, J.C.; et al. Neurochemical Markers in CSF of Adolescent and Adult SMA Patients Undergoing Nusinersen Treatment. Ther. Adv. Neurol. Disord. 2019, 12, 175628641984605, doi:10.1177/1756286419846058.
50. Faravelli, I.; Meneri, M.; Saccomanno, D.; Velardo, D.; Abati, E.; Gagliardi, D.; Parente, V.; Petrozzi, L.; Ronchi, D.; Stocchetti, N.; et al. Nusinersen Treatment and Cerebrospinal Fluid Neurofilaments: An Explorative Study on Spinal Muscular Atrophy Type 3 Patients. J. Cell. Mol. Med. 2020, 24, 3034–3039, doi:10.1111/jcmm.14939.
51. Badina, M.; Sporea, C.; Bejan, G.C.; Mirea, A.; Ion, D.A. The Dynamic of Changes of PNFH Levels in the CSF Compared with the Motor Scales' Scores during Three Years of Nusinersen Treatment in Children with Spinal Muscular Atrophy Types 2 and 3. Balneo PRM Res. J. 2024, 15, 643, doi:10.12680/balneo.2024.643.
52. Sarıkaya Uzan, G.; Paketçi, C.; Günay, Ç.; Edem, P.; Özsoy, Ö.; Hız Kurul, S.; Yiş, U. The Effect of Nusinersen Therapy on Laboratory Parameters of Patients with Spinal Muscular Atrophy. Neuropediatrics 2022, 53, 321–329, doi:10.1055/s-0042-1750719.
53. Stolte, B.; Nonnemacher, M.; Kizina, K.; Bolz, S.; Totzeck, A.; Thimm, A.; Wagner, B.; Deuschl, C.; Kleinschnitz, C.; Hagenacker, T. Nusinersen Treatment in Adult Patients with Spinal Muscular Atrophy: A Safety Analysis of Laboratory Parameters. J. Neurol. 2021, 268, 4667–4679, doi:10.1007/s00415-021-10569-8.
54. Silva, P. Serum Creatinine Levels Reflect Disease Severity in Muscular Dystrophy Patients Available online: https://musculardystrophynews.com/news/serum-creatinine-levels-reflect-disease-severity-in-muscular-dystrophy-patients/ (accessed on 5 January 2024).
55. Mirea, A.; Leanca, M.C.; Onose, G.; Sporea, C.; Padure, L.; Shelby, E.-S.; Dima, V.; Daia, C. Physical Therapy and Nusinersen Impact on Spinal Muscular Atrophy Rehabilitative Outcome. Front. Biosci. 2022, 27, 179, doi:10.31083/j.fbl2706179.