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Research article

Predictive Clinical-Biological Markers Over the First 3 Years of Nusinersen Treatment in SMA Type 1 Patients

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Abstract: Werdnig-Hoffmann disease, or type 1 Spinal Muscular Atrophy (SMA), is caused by insufficient SMN protein synthesis due to a genetic defect. Symptoms appear within the first 6 months of life, and without ventilatory support, life expectancy averages 2 years. This study aimed to monitor pNF-H neurofilament levels in cerebrospinal fluid (CSF) and serum, serum creatinine, and motor performance during nusinersen treatment to evaluate pNF-H as a predictor of motor outcomes. Biological samples and clinical outcomes from 34 participants were analyzed at 6 months, 1 year, 2 years, and 3 years post-treatment initiation. Most patients showed favorable outcomes, with improved motor assessment scores, increased serum creatinine, and decreased pNF-H levels in CSF and serum. Higher baseline pNF-H in CSF was linked to fewer SMN2 gene copies. The largest pNF-H decrease occurred during the nusinersen loading period, stabilizing at low levels through maintenance. Smaller changes in pNF-H from baseline correlated with better motor outcomes and higher serum creatinine at 2 and 3 years. Nusinersen treatment reduced pNF-H levels, reflecting decreased neuronal degradation, increased serum creatinine due to enhanced muscle activity, and improved motor function. High baseline pNF-H in SMA type 1 may indicate a poorer prognosis for motor improvement.

Keywords: spinal muscular atrophy; Spinraza nusinersen; pNF-H neurofilaments; cerebrospinal fluid; creatinine; motor evolution; predictive markers; neurodegenerative disease; genetic mutation SMN

1. Introduction

Spinal amyotrophy, also known as spinal muscular atrophy (SMA), is a rare neurodegenerative disease with autosomal recessive inheritance. It is caused by insufficient synthesis of the SMN protein, which is essential for motor neuron survival. In over 95% of cases, this deficiency is due to a deletion or mutation in the 5q13 region of the SMN1 gene, which encodes this protein [1].

In the absence of the SMN1 gene, the homologous SMN2 gene compensates, though it differs structurally by a single nucleotide substitution (T to C at position 840). This change results in the production of an incomplete protein that is quickly degraded in cells. Each copy of the SMN2 gene can produce approximately 10% of the SMN protein needed for normal functioning. However, the required amount varies between cells and tissues, leading to phenotypic variation [2–4].

The consequence of insufficient SMN protein synthesis is the gradual degradation of motor neurons in the anterior horns of the spinal cord, resulting in progressive muscle weakness and skeletal muscle atrophy. This degeneration is accompanied by respiratory and digestive complications which, over time and in the absence of

adequate treatment, can lead to respiratory failure, paralysis, and even death in severe cases [1,5].

The Austrian neurologist Guido Werdnig was the first to describe spinal muscular atrophy in 1891, in two siblings. Between 1893 and 1900, the German neurologist Johann Hoffmann documented seven additional cases, also in children.

Werdnig-Hoffmann disease, or SMA type 1, classified based on the age of onset, is the most common form of spinal muscular atrophy, accounting for about 58% of pediatric cases [6]. Symptoms appear within the first 6 months of life, and patients present with a "floppy infant" appearance due to severe hypotonia, predominantly axial muscle weakness, and diminished deep tendon reflexes [7–10]. Other symptoms include weak crying, hypoventilation, paradoxical breathing, ineffective cough, difficulties in swallowing and sucking, and tongue fasciculations [11,12]. These children do not acquire the ability to walk, cannot maintain a sitting position without support, and sometimes lack head control [9,13]. Genetically, most of these patients have a single SMN2 gene copy for subtype 1A and two SMN2 copies for subtypes 1B and 1C. Without ventilatory support, they often do not survive beyond the first 2 years of life without disease-modifying treatment [14,15].

The approval of Nusinersen (Spinraza) in December 2016 in the United States, in June 2017 by the European Medicines Agency, and in March 2018 in Romania has altered the disease's natural progression, extending life expectancy and improving motor skills. Outcomes are particularly influenced by the age at which treatment begins and the intensity of supportive therapies, including physiotherapy, physical therapy, and occupational therapy [16–18].

Nusinersen is an antisense oligonucleotide targeting the SMN2 gene, designed to prevent premature splicing of messenger RNA. This process enables the production of a full transcript, inducing the synthesis of a normally functioning SMN protein [19–22]. It is administered intrathecally, typically through lumbar puncture, with an initial loading period followed by a maintenance phase, during which doses are administered once every four months for life [20].

The progress of patients undergoing treatment is monitored through physical performance assessments using motor function evaluation scales, some of which are specifically designed for SMA patients (e.g., CHOP-INTEND, HINE, HFMSE, RULM) [23–26].

Neurofilaments are protein structures specific to neurons, serving structural functions or facilitating nerve impulse conduction. They appear in the cerebrospinal fluid (CSF) and, subsequently, in the blood in cases of acute conditions or chronic neurodegenerative diseases that involve neuron damage or destruction [27–30].

Based on physical, chemical, and, more recently, genetic characteristics, neurofilaments have been differentiated into five subunits. The pNF-H subunit is the largest, most abundant, and most stable, due to extensive phosphorylation [28,31–37].

We hypothesize that in SMA type 1, the younger the age at treatment initiation, the better the recovery. Neurofilament levels are expected to decrease as the destruction of motor neurons slows or stops due to the restoration of SMN protein, while muscle strength and motor performance improve. These improvements would be reflected by higher serum creatinine levels and increased scores on motor scales.

The aim of this study was to monitor pNF-H neurofilament levels in CSF and serum at 6 months, 1 year, 2 years, and 3 years after the initiation of nusinersen treatment in patients with SMA type 1. Additionally, we assessed changes in pNF-H levels relative to serum creatinine and motor scale performance over time, considering the age at treatment initiation and correlations among the studied parameters.

2. Materials and Methods

The batch of the present study followed the evolution of patients diagnosed with SMA type 1 according to the age of onset of clinical symptoms and treated in National

Teaching Center for Children's Neurorehabilitation “Dr. Nicolae Robanescu" between October 2018 and November 2023 through the National Program for Rare Diseases - Treatment with Nusinersen - the first disease-modifying treatment approved both in Romania and worldwide.

The legal relatives of the patients signed the participation consent, and the study was approved by the Ethics Committee of the CNCRNC "Dr. Nicolae Robănescu" (agreement number 7464/01.10.2018) in accordance with local regulations and the World Medical Association Declaration of Helsinki, revised in 2000, Edinburgh.

The study included 34 children, diagnosed, evaluated, and monitored by specialized staff experienced in managing SMA in pediatric patients. Clinical and laboratory analyses were performed by the same personnel using the same analyzers to minimize bias. Clinical and paraclinical data were extracted from patient observation sheets and processed using IBM SPSS Statistics 24 and Microsoft Excel 2021.

In this study, the following parameters were analyzed: pNF-H neurofilament levels in CSF and serum samples (measured by the ELISA method as an indicator of neuronal destruction), serum creatinine levels (reflecting muscle activity), and scores on motor scales (for functional evaluation). Serum sampling and motor assessments were conducted before each intrathecal administration of nusinersen. During this procedure, CSF was initially collected under sterile conditions, following the drug administration protocol [38].

Motor evaluation was conducted using the CHOP-INTEND scale, specifically designed for SMA type 1 patients aged between 1.4 and 38 months. This scale has a maximum score of 64 points, based on the execution of 16 tasks, each rated on a 4-point scale [39,40].

3. Results

In this study, CSF and serum samples were analyzed to determine pNF-H neurofilament levels using the ELISA method. Additionally, we monitored the progression of neurofilaments, serum creatinine levels, and motor scale scores over time.

The analyzed time points were as follows:

T0 – before treatment initiation (first dose, I1)

T1 – before the maintenance period began (fifth dose, I5, 6 months after treatment initiation)

T2 – sixth dose (I6), 8 months after initiation

T3, T4, and T5 – at one, two, and three years post-initiation, corresponding to doses 7 (I7), 10 (I10), and 13 (I13), respectively.

Of the 34 participants, aged between 0.67 and 46 months at the start of treatment, female patients were slightly more prevalent (56%). Regarding the number of SMN2 gene copies, over three-quarters of patients (76%) had 2 copies, while 8 patients (24%) had 3 copies, all classified as SMA type 1 based on the age of symptom onset.

The youngest patient was a male, aged 0.67 months at treatment initiation, diagnosed with SMA type 1 with 2 SMN2 gene copies. The oldest patient, also male and with 2 copies of the SMN2 gene, was 46 months old at initiation, highlighting the presence of additional factors influencing the clinical presentation beyond the SMN2 gene copy number.

Table 1 presents the values of parameters recorded at the start of treatment, including the minimum and maximum levels of pNF-H neurofilaments in CSF and serum, serum creatinine, and motor scale scores, categorized by the number of SMN2 copies.

Table 1. Biomarker levels and functional scale scores at the initiation of treatment

		SMA type 1	
	Total	**2 SMN2 copies**	**3 SMN2 copies**
pNF-H in CSF (ng/ml) Min ÷ Max	0,039÷3,321	0,039÷3,321	0,057÷0,352
pNF-H in serum (ng/ml) Min ÷ Max	0,032÷4,595	0,217÷4,595	0,032÷1,450
Serum creatinine (mg/dl) Min ÷ Max	0,20÷0,44	0,20÷0,44	0,29÷0,44
CHOP-INTEND Min ÷ Max	7÷57	7÷50	24÷57

Prior to treatment initiation, the highest CSF pNF-H neurofilament level (3.321 ng/ml) was observed in a 3.63-month-old female patient with 2 SMN2 copies, while the lowest level (0.039 ng/ml) was found in a 9-month-old female patient, also with 2 SMN2 copies.

In serum, the highest pNF-H neurofilament level (4.595 ng/ml) was recorded in a 55.03-month-old female patient with 2 SMN2 copies, while the lowest level (0.032 ng/ml) was observed in a 10-month-old female patient with 3 SMN2 copies.

For creatinine, the lowest value (0.20 mg/dl) was recorded in a 24-month-old female patient with 2 SMN2 copies, while the highest value (0.44 mg/dl) was observed in two patients with 3 SMN2 copies—a 9-month-old female and a 10-month-old male.

At treatment initiation, all patients showed the highest pNF-H neurofilament levels in CSF, regardless of the number of SMN2 copies. During the subsequent three-year maintenance period, CSF neurofilament levels remained below 0.401 ng/ml for most patients. Exceptions were observed in two patients with 2 SMN2 copies, who had elevated levels at one and two years of treatment, likely due to acute events between injections; however, their levels returned to group norms in subsequent assessments.

The lowest CSF pNF-H levels were observed at three years of treatment for all patients included in the study (Figure 1).

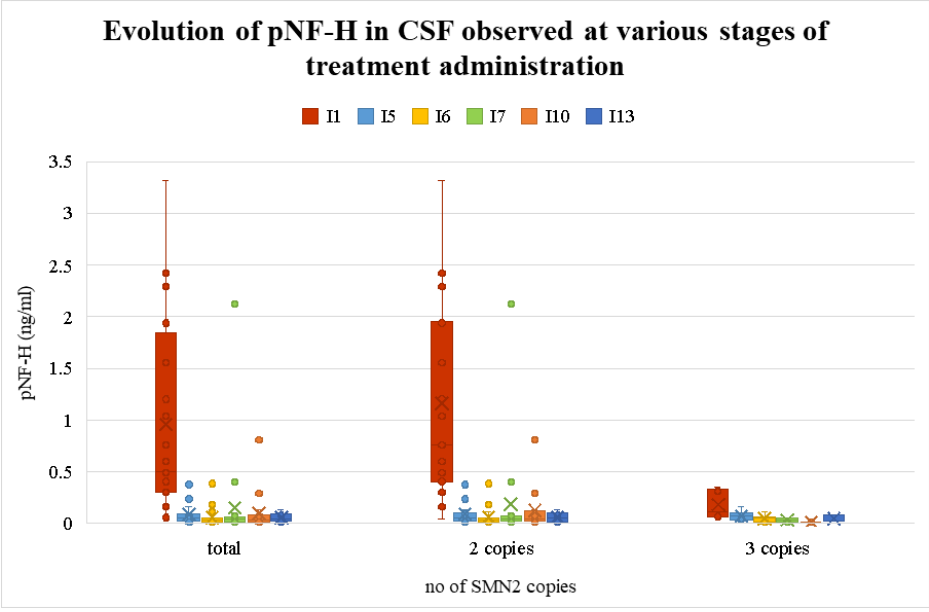

Figure 1. Evolution of pNF-H neurofilament levels in CSF at various treatment time points, categorized by the number of SMN2 gene copies.

For pNF-H levels in serum, a similar trend to that in CSF was observed, with the highest values at baseline in patients with 2 SMN2 copies. These levels generally decreased during nusinersen treatment to below 0.448 ng/ml. Exceptions included two patients with severe clinical forms, whose serum pNF-H levels decreased more slowly; at the 6-month evaluation, their levels were 7.280 and 0.825 ng/ml, respectively (Figure 2).

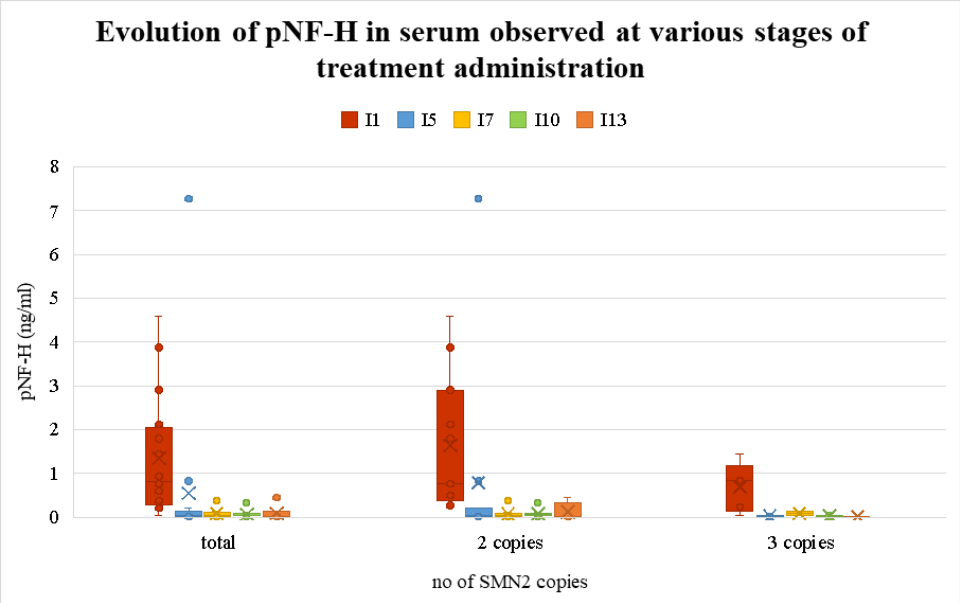

Figure 2. Evolution of pNF-H neurofilament levels in serum at various treatment time points, categorized by the number of SMN2 copies

Serum creatinine levels increased over the three-year treatment period compared to baseline values across all patient groups, though a decrease was observed at the two-year mark (Figure 3).

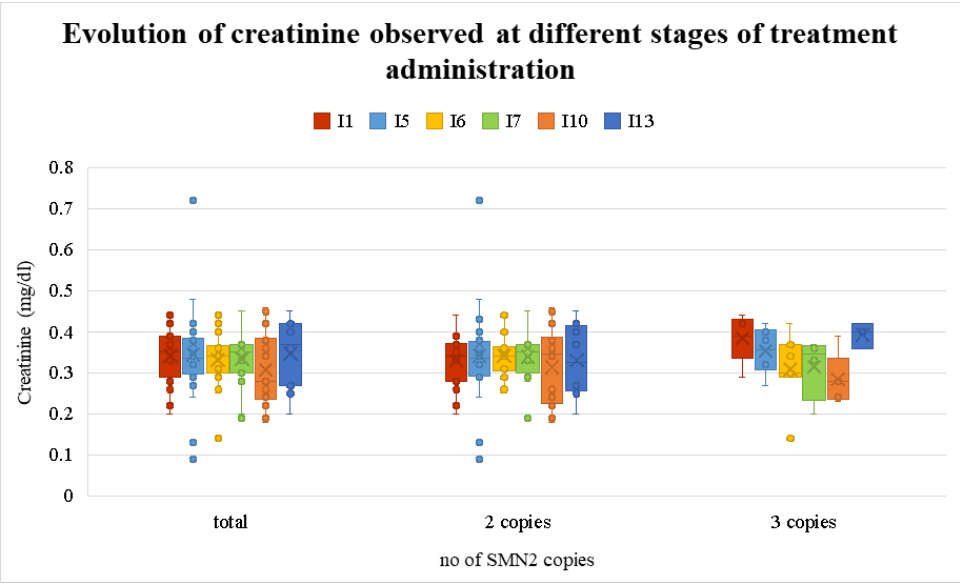

Figure 3. The evolution of serum creatinine level at different times of treatment according to the number of SMN2 copies

Motor scores showed significant improvement over the three-year treatment period, with patients achieving higher scores compared to their initial baseline, indicating enhanced motor function and responsiveness to treatment.

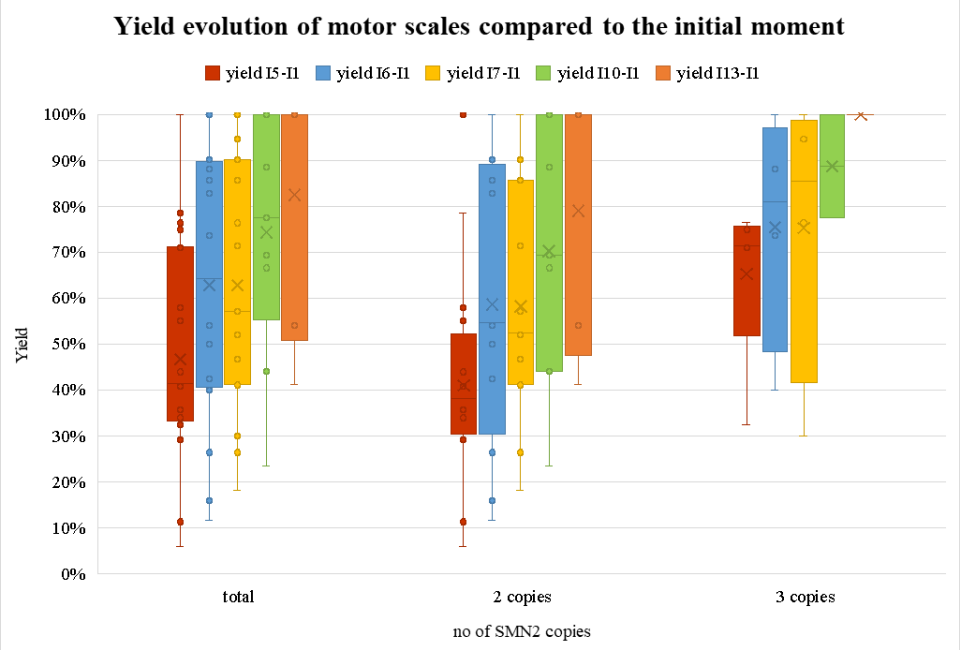

Figure 4. The yield evolution of motor scales compared to initial moment at different times of treatment according to the number of SMN2 copies

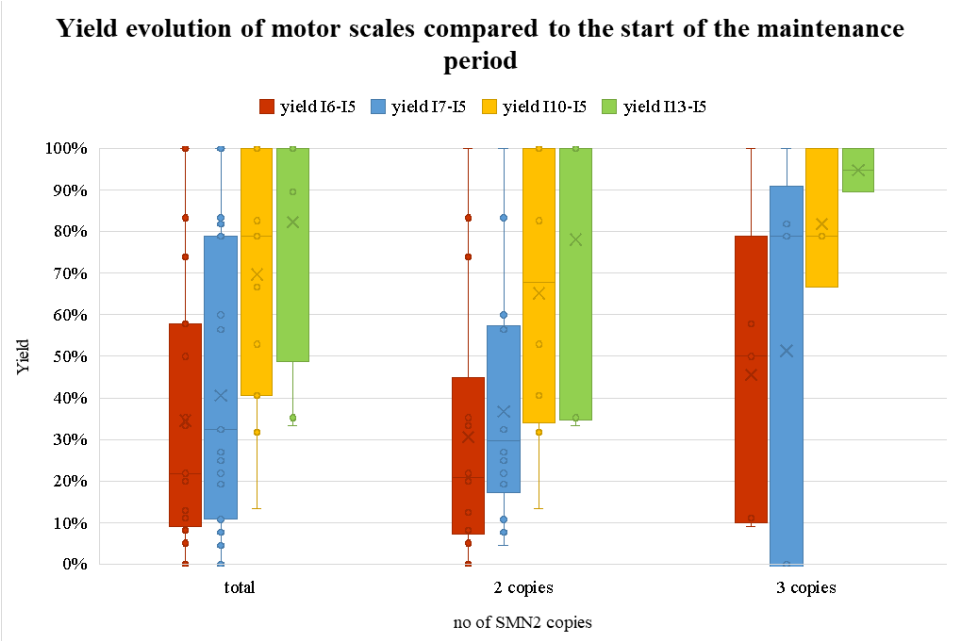

Figure 5. The yield evolution of motor scales compared to the start of maintenance period at different times of treatment according to the number of SMN2 copies

The level of pNF-H neurofilaments in CSF correlated weakly negatively with the number of copies of the SMN2 gene (r = -0.434), moderately positively with the level of pNF-H neurofilaments in CSF from dose I6 (r = 0.532) and strongly positively (r = 0.969) with the evolution of the level of neurofilaments in the serum during the maintenance period of the first year of treatment (between the injection of doses I7 and I5) where the difference between the pNF-H values in the serum was between a minimum of 0.117 ng/ml and a maximum of 0.717 ng/ml.

Regarding the motor evolution, the level of pNF-H in the CSF correlated strongly negatively with the CHOP-INTEND score after three years of treatment (r = -0.840), weakly negatively with the performance calculated until before the second maintenance dose – between I1 and I6 (r = -0.482), strongly positive and very strongly

positive with the yield obtained after two years (I10), respectively 3 years (I13) of treatment (r = -0.735 and -0.992), where the average yield after two years was 76.9%, and after three years 82.5% of the maximum possible value of the evolution on the scale of maximum 64 points.

SMN2 gene copy number correlated in weakly positive with CHOP-INTEND score at baseline (r = 0.430) and at the beginning of the maintenance period (r = 0.434). The number of SMN2 copies was moderately negatively correlated with the percentage evolution of the CSF pNF-H level in the first year of treatment in the maintenance period (I5, I6 and I7) relative to the value before the start of treatment (r = -0.531 and -0.519, respectively and -0.540).

The age of the patients at initiation was moderately positively correlated (r = 0.606) with the score obtained on the CHOP-INTEND scale at the time of treatment initiation, where the values between 7 and 57 points had an average value of 28.45 out of the maximum possible total of 64 points . Age was also weakly positively correlated (r = 0.423) with neurofilament levels at the time of the 6th dose, strongly positively with the change in serum pNF-H levels during the maintenance period of the first year of treatment (r = 0.819) and very strongly positive with the difference in the evolution of the level of neurofilaments in the CSF from the maintenance period of the three years of treatment (between I13 and I5, r = 0.908).

The level of pNF-H in the serum before the initiation of treatment correlated moderately negatively (r = -0.572) with the percentage evolution of the level in the CSF between the first two doses of the maintenance period (between I6 and I5) relative to the initial value and moderately positively (r = 0.626) with the serum creatinine level before the second maintenance dose (I6), whose values were between 0.44 mg/dl (for two female children with two copies of the SMN2 gene and age 1.3 and 5.03 months at treatment initiation) and 0.14 mg/dl (for a female patient with 3 copies of the SMN2 gene, aged 7 months at initiation).

Serum creatinine level was moderately negatively correlated with CSF neurofilament pNF-H level at the time of administration of the second (I6) and third (I7) maintenance dose of nusinersen (r = -0.664 and -0.688), weak negative with the evolution of pNF-H in the CSF during the loading period (r = -0.460) and very strong and negative with the evolution of the pNF-H level in the serum during the maintenance period of the first year of treatment (r = -0.930).

The level of scores on the CHOP-INTEND scale before the initiation of treatment correlated strongly and positively with those obtained at the initiation of maintenance treatment and at one year of treatment (r = 0.836 and 0.714, respectively) and moderately positively with those at dose I6 and from two years of treatment (r = 0.655, respectively 0.680).

The CSF pNF-H neurofilament level at the start of maintenance treatment (I5) ranged from a low of 0.009 ng/ml (for a male patient with 3 copies of the SMN2 gene and 10 months at baseline and a female patient with 2 SMN2 gene copies and 6.5 months at baseline) and a maximum of 0.389 ng/ml (for a patient with 2 SMN2 copies, aged 7 months at baseline). The level of pNF-H in the CSF was strongly positively correlated (r = 0.825) with the variation of the level of pNF-H in the serum in the first year of treatment and strongly negatively with the performance obtained on the motor assessment scales in the first two years of treatment (r = -0.733).

Serum pNF-H level at the beginning of maintenance treatment correlated strongly negatively (r = -0.720) with the evolution of serum creatinine level in the first year of treatment and very strongly negatively (r = -0.956) with performance on motor functional scales in the first two years of treatment.

The score obtained at the beginning of maintenance treatment (I5) on the CHOP-INTEND scale, with a minimum of 25 points (for a child with 2 SMN2 copies and 6 months at initiation) and a possible maximum of 64 points (for a child also with 2 copies of the SMN2 gene but 1.3 months at initiation) was strongly positively correlated with the scores obtained at the following moments analyzed: I6 (r = 0.848), I7 (r

= 0.896), I10 (r = 0.837), I13 (r = 0.750) and with the yield obtained in the first year of treatment – between I7 and I1 (r = 0.804) and moderately positive with the yield obtained until the I6 injection (r = 0.686).

At injection 6, the CSF pNF-H level, ranging from a low of 0.009 ng/ml for 5 patients in the group (one with 3 SMN2 copies and 10 months at baseline and the rest with 2 SMN2 copies, aged 5 to 6.5 months at baseline) and a maximum of 0.387 ng/ml (for a patient with 2 SMN2 copies and 24 months at baseline), was moderately positively correlated (r = 0.513) with the change in CSF pNF-H level during the nusinersen loading period and very strongly positive with the variation of the level during the maintenance period up to three years of treatment – between I13 and I5 (r = 0.935) and with the variation of the serum pNF-H level during the maintenance period of the first year of treatment – between I7 and I5 (r = 0.988) and only moderately negative with the variation of serum creatinine level from the first year of treatment – between I7 and I1 (r = -0.538). For the level of pNF-H in the CSF one year after initiation of treatment (I7), a minimum level of 0.009 ng/ml was obtained for a patient with 3 SMN2 copies and 7 months at initiation and a maximum of 0.401 ng/ml for a patient with 2 SMN2 copies and 24 months at initiation. The level of pNF-H in the CSF at one year of treatment correlated very strongly positively with the variation of the level of pNF-H in the serum during the maintenance period of the first year of treatment (between I7 and I5) with a coefficient r = 0.984, moderately negative with the variation serum creatinine level in the first year of treatment (r = -0.595) and moderately negative with performance on motor scales in the first two years of treatment (r = -0.691).

The level of pNF-H in the serum at one year of treatment (I7), with values between 0.016 ng/ml and 0.379 ng/ml, was strongly negatively correlated (r = -0.782) with the variation of the level of pNF-H in the CSF during the period of maintenance from the first year of treatment (between I5 and I7).

Serum creatinine at one year of treatment had values between 0.19 and 0.45 mg/dl and correlated moderately negatively with the variation in the level of pNF-H in the CSF during the loading period (r = -0.533), strongly positively with the variation in the level pNF-H in serum in the first year of treatment (r = 0.774), with the percentage of this variation relative to the initial value in serum (r = 0.827) and with the percentage of variation in the level of pNF-H in serum during the maintenance period of the first year of treatment relative to baseline serum value (r = 0.846).

The CHOP-INTEND score at one year of treatment ranged from 28 to 64 points, with a mean of 49 points, and was positively and strongly or very strongly correlated with the score obtained at two and three years of treatment (r = 0.840, respectively 0.910) and strong or very strong with the performance obtained on the motor scales during the loading period and in the first year of treatment (r = 0.754 and 0.924).

The level of pNF-H neurofilaments in the CSF at two years of treatment (I10) was between a minimum of 0.009 ng/ml and a maximum of 0.809 ng/ml and was strongly negatively correlated with performance on the motor scales obtained in two and three years of treatment (r = -0.732 and -0.885), while the serum level of pNF-H after two years of treatment correlated very strongly positively (r = 0.969) with the level obtained after three years of treatment.

The serum creatinine level after two years of treatment was between 0.18 mg/dl and 0.46 mg/dl, for patients with two copies of the SMN2 gene, but with an age at the initiation of treatment of 6 and 1.3 months, respectively, and moderately positively correlated with the CHOP-INTEND score at two years of treatment (r = 0.579), strongly positively correlated with the CSF pNF-H level at three years of treatment (r = 0.787), moderately negatively correlated with the variation of the relative CSF pNF-H level at the initial level during the maintenance period of the first year of treatment (r = -0.546), very strongly negative with the variation of pNF-H level in the CSF relative to the initial level in the three years of maintenance treatment (r = -0.965) and with the variation of serum pNF-H level relative to the initial value for the first year of treatment (r = -0.916). The score obtained on the CHOP-INTEND scale after

two years of treatment, between 29 and 64 points, with an average of 50.76 points, correlated very strongly positively with the score obtained after three years of treatment, between 32 and 64 points, with an average of 53.71 points (r = 0.925), and compared to the evolution of the pNF-H level in the CSF between the studied moments compared to the initial level, negative and strong correlations were highlighted for injections I6 (r = -0.762), I7 (r = -0.754) and I10 (r = -0.735) and a moderate correlation for I5 (r = -0.679). Compared to the yield obtained up to the studied moments, the correlations were positive and strong, with a coefficient between 0.806 and 0.833 (for I5-I1, I6-I1, I7-I1 and I10-I1) and a very strong correlation for I13-I1 (r = 0.937).

At three years of treatment, the CHOP-INTEND score was strongly negatively correlated with the variation of the CSF pNF-H level between the initial value and the value at the time studied (r = -0.869 for I5, -0.895 for I6, -0.892 for I7 and -0.849 for I10). The variation of pNF-H level in CSF for the loading period with nusinersen correlated positively and very strongly with the variation of pNF-H level in serum during the maintenance period of the first year of treatment – between I7 and I5 (r = 0.943) and from the first two years of treatment – between I10 and I1 (r = 0.901), moderately negative with the performance on the motor scales of the same period – between I10 and I1 (r = -0.656) and very strongly negative with the performance of the three years of treatment – between I13 and I1 (r = -0.987).

The change in the level of pNF-H in the first year of treatment in absolute value, with a mean of 0.839 ng/ml and a percentage of 79.5% relative to the initial value, was moderately negatively correlated with the performance on the motor scales in the first two years (r = -0.692) and very strongly negative with the return of the three years of treatment (r = -0.982), as well as the variation of the pNF-H level in the first two years of treatment in absolute value, with an average of 0.830 ng/ml and a percentage of 18.5% relative to the initial value, which correlated strongly negatively with the performance on the motor scales in the first two years (r = -0.721) and very strongly negatively with the performance in the three years of treatment (r = -0.992).

The variation of the serum creatinine level during the loading period in absolute values (between I1 and I5), with an average of 0.81 mg/dl, was strongly negatively correlated with the variation of the serum creatinine level between the first two doses of the maintenance period – between I5 and I6 (r = -0.790) and with the variation of the serum creatinine level during the maintenance period of the first year of treatment – between I5 and I7 (r = -0.869) and moderately negative with the variation of the serum creatinine level during the maintenance treatment period of the first two years of treatment – between I5 and I10 (r = -0.682).

4. Discussion

After analyzing the results obtained following the administration of nusinersen treatment for three years, the majority of patients had a favorable evolution. Motor rating scale scores improved due to increased muscle mobility and strength, serum creatinine increased due to more intense muscle activity, and CSF and serum pNF-H neurofilament levels decreased due to decreased degradation motor neurons due to the increase in the amount of SMN protein necessary for the survival of these neurons, similar to the results of other studies in the specialized literature [18,41–44].

The most important decrease in the pNF-H level in the CSF was observed during the loading period with nusinersen, in the first 6 months after the initiation of treatment, and in the maintenance period the pNF-H level in the CSF was for most patients at values below a level basal probably due to normal neuronal metabolism [45,46].

The serum pNF-H level followed the same course of gradual decline during treatment and stabilized below a trough value for most patients at a slower rate than the CSF level.

Serum creatinine level increased with increasing muscle activity and was accompanied by improvement in scores on motor rating scales [47–49].

The level of pNF-H neurofilaments in the CSF at the start of treatment had higher values the lower the number of copies of the SMN2 gene, so in the most severe forms of the disease and as close to the onset of symptoms, when the number of viable motor neurons was higher, as was the SMN protein requirement for their survival [45,46,50,51].

Determination of a higher CSF pNF-H level at baseline led to the observation of higher serum pNF-H levels and evolved with a more pronounced decrease during treatment [52,53].

Constant correlations for assessing the evolution of patients under nusinersen treatment were observed both between the absolute values of the pNF-H level in the CSF and the other studied parameters (age, the number of copies of the SMN2 gene, the scores obtained on the motor scales, the levels of serum creatinine and pNF-H neurofilaments in serum), especially between the variations of the values obtained in different treatment periods compared to the values at the beginning of the treatment or at the beginning of the maintenance period, in absolute values or in values relative to the initial value [18,53–55].

A younger age at treatment initiation was associated with a higher CHOP-INTEND score, a smaller change in CSF pNF-H level during the maintenance period and serum pNF-H level during the maintenance period from the first year of treatment.

The higher the number of SMN2 copies, the higher the scores obtained on the motor scales at initiation and at the beginning of maintenance treatment, and the variation of pNF-H level in CSF during the maintenance period from the first year of treatment relative to the initial value was lower.

A higher CSF neurofilament pNF-H level at treatment initiation for these patients correlated with a lower CHOP-INTEND score at 6 months, one year, two and three years of treatment. Lower yield at two years of treatment was associated with higher CSF pNF-H levels at 6 months, one year and two years of treatment.

A smaller CSF pNF-H change from baseline to 6 months, one year, and two years of treatment was associated with better outcomes at both two and three years of treatment. The motor score for this group of patients with SMA type 1, the most severe type of the disease, obtained after two years of treatment, correlated inversely proportionally with all the variations of the pNF-H level in the CSF compared to the initial level for the studied moments (during the first year of maintenance treatment, at two and three years of treatment).

Greater variation in serum creatinine level during the loading period was found to be less variation in serum creatinine level during the maintenance period at one year and two years of treatment.

Achieving better returns at each time point during the maintenance treatment period was associated with a better CHOP-INTEND scale score at two years of treatment.

Limitation

The main impediment encountered in these study was the short-term approval of the disease-modifying treatment, so that the study group had a wide palette of ages at the initiation of the treatment, the timing being primarily related to the availability of the therapy and not to the onset of symptoms.

Individual variations in own metabolism, symptomatology polymorphism and the application of adjunctive kinesitherapy and physiotherapy treatments require caution in the interpretation of the results.

5. Conclusions

Treatment with nusinersen brought benefits to most patients, evidenced paraclinically by the decrease in the level of pNF-H neurofilaments in the CSF and serum as a result of stopping the neuronal degradation due to the lack of SMN protein, by increasing the level of serum creatinine as a result of the intensification of muscle activity or clinically by increasing the scores obtained on the motor scales due to the increase in mobility and muscle strength.

After treatment, the level of pNF-H neurofilaments in the CSF decreased gradually, most during the loading period, and then remained constantly below a value probably originating from normal neuronal metabolism in situations where no factors appeared to cause new neuronal damage. In the case of the occurrence of such events, the level of neurofilaments in the CSF increased, and then returned to the values before the respective events and evolved similarly to that of other patients in the same study group.

Treatment instituted early in the course of the disease was associated with better clinical outcomes, higher baseline motor performance, and a lower number of degraded motor neurons due to the lack of SMN protein, according to studies in the literature.

In the most severe form of the disease – SMA type 1, obtaining a low level of pNF-H in the CSF at the start of treatment could be considered a positive predictive factor for the returns on the motor scales throughout the treatment, and obtaining low values of of pNF-H neurofilament levels in CSF throughout treatment suggests a better outcome at two years of treatment.

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