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Research article

Evolution of Functional and Paraclinical Markers as Predictive Factors in Pediatric Late-Onset SMA Under Nusinersen Treatment: The Role of CSF pNF-H

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Abstract: Spinal Muscular Atrophy (SMA) is a rare neurodegenerative disease caused by insufficient synthesis of SMN protein, characterized by progressive muscle weakness, atrophy, and complications affecting the respiratory and digestive systems. Disease severity tends to be greater when symptoms manifest at an earlier age. Since 2016, the FDA-approved drug nusinersen has provided a disease-modifying treatment option. Identifying predictive factors for patient outcomes over time remains essential. This retrospective study analyzed clinical and biological parameters in 42 patients (ages 13–215 months) with SMA types 2 and 3 over the first three years of nusinersen treatment. We assessed pNF-H levels in CSF and serum—neuronal proteins associated with neurodegeneration—as well as serum creatinine levels, a marker of muscle activity, and motor skill scores to evaluate pNF-H's potential as a predictor of motor development. Elevated pNF-H levels were associated with a lower SMN2 gene copy number and more recent disease onset. Following nusinersen treatment, pNF-H levels stabilized at low values, likely due to basal metabolic activity. In SMA types 2 and 3, higher baseline CSF pNF-H levels correlated with improved performance over time. Additionally, higher serum creatinine levels and smaller changes in pNF-H during the loading phase or various periods of maintenance treatment were associated with better motor development outcomes at two and three years of treatment.

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

1. Introduction

Spinal Muscular Atrophy (SMA) is a rare genetic disease with autosomal recessive transmission. It is characterized by the progressive degradation of motor neurons in the anterior horns of the spinal cord, caused by insufficient synthesis of the SMN protein, which is essential for motor neuron survival. The disease progresses with increasing muscle weakness and subsequent atrophy, often accompanied by respiratory and digestive complications that can lead to death in severe cases [1].

Under normal conditions, the SMN protein is synthesized by the SMN1 gene located in the 5q13 region. However, in cases of biallelic deletions or mutations, this function is compensated by the paralogous SMN2 gene, which produces a shorter, less stable, and less functionally efficient protein [2–5]. Each copy of the SMN2 gene synthesizes approximately 10% of the body's required SMN protein, and the number of SMN2 copies significantly influences the severity of clinical symptoms [6,7].

Based on the age of symptom onset, SMA is classified into five types. Type 0 is the rarest and most severe form, with symptoms appearing in utero, typically associated with a single copy of the SMN2 gene and a life expectancy of around 6 months. Type 1 presents within the first 6 months of life; affected infants have a "floppy" appearance (floppy infant syndrome), cannot sit unsupported, and, without ventilatory support, usually do not survive beyond 2 years. In type 2 SMA (Dubowitz disease), which accounts for about 29% of pediatric cases, symptoms typically appear between 6 and 18 months of age [8,9]. Patients with this type usually have 2 or 3 copies of the SMN2 gene, can sit independently, but cannot walk without support. They experience varying degrees of muscle weakness and generalized hypotonia, particularly in proximal muscles, along with lingual fasciculations and diminished deep tendon reflexes [10,11]. Over time, additional complications arise, including bone deformities (especially of the ribcage), scoliosis, joint contractures, respiratory and swallowing difficulties, and an inefficient cough. Life expectancy is longer in type 2 SMA, with many patients reaching adulthood [12]. Type 3 SMA (Kugelberg-Welander disease) accounts for about 13% of pediatric cases and is typically diagnosed after 18 months, though sometimes not until adolescence, when the first symptoms appear [8,9,13,14]. Patients with this type often have 3, 4, or more copies of the SMN2 gene. The disease progresses with the gradual loss, to varying degrees, of previously acquired skills, although life expectancy is not impacted [12]. Proximal muscle weakness predominantly affects the lower limbs, initially leading to difficulties in climbing stairs, a tendency for frequent falls, and mild gait disturbances, potentially progressing to the loss of the ability to walk. However, some patients retain the ability to walk without support throughout their lives [12,15–18]. In SMA type 4, symptoms typically appear in adulthood, usually after the age of 30, and life expectancy remains normal [14].

Motor performance in SMA patients is assessed using scales specifically created and validated according to clinical characteristics, severity, age, and disease type. These scales evaluate factors such as voluntary and reflex movements, posture, walking ability (with or without support), and resistance to physical effort [19,20].

Nusinersen (Spinraza), approved in December 2016, was the first drug available for the treatment of SMA. It is administered intrathecally via lumbar puncture, following a protocol that includes an initial loading period (4 doses) and a maintenance phase with doses given once every four months for life [21]. Nusinersen is an antisense oligonucleotide targeting the SMN2 gene, preventing premature splicing of the messenger RNA and enabling the production of a full-length transcript, which synthesizes a normal-length, functional SMN protein [21–24].

Neurofilaments are neuron-specific heteroproteins that appear in cerebrospinal fluid (CSF) and subsequently in peripheral blood due to neuronal damage. They exist in five subunits, with pNF-H representing the phosphorylated form of the largest, most stable subunit. This subunit is abundant in neurons, especially in the axons where it is most concentrated [2,25–31].

Various studies on neurodegenerative diseases associated with neuronal damage—such as Parkinson's disease, Alzheimer's disease, Charcot-Marie-Tooth disease, frontotemporal dementia, vascular dementia, Lewy body dementia, amyotrophic lateral sclerosis, multiple sclerosis, toxic or giant axon neuropathy, and severe burns—have shown that pNF-H levels in cerebrospinal fluid (CSF) are directly proportional to the extent of neuronal damage, reflecting disease severity, progression rate, and treatment efficacy [2,25–27,32,33].

In patients with SMA types 2 and 3, we hypothesize that the highest pNF-H levels are observed in the most severe disease forms and during periods of marked progression. This is especially true when a large number of viable neurons are affected, which tends to occur in patients with a shorter disease history, less advanced motor impairment, and better potential for recovery. The impact of nusinersen treatment may be demonstrated by reductions in pNF-H levels in CSF and serum—indicating

reduced neuronal degradation, increases in serum creatinine levels—reflecting heightened muscle activity, and improvements in motor scale scores—indicating enhanced motor function.

This study aimed to determine pNF-H levels in CSF and serum versus changes in serum creatinine and motor scale scores following nusinersen treatment to determine the role of pNF-H level monitoring as a predictor of motor development over time according to the correlations of the values and variations of these parameters in different time intervals.

2. Materials and Methods

42 patients diagnosed with SMA type 2 or 3 and treated with Nusinersen between October 2018 and October 2023 participated in this retrospective monocentric study. The study was carried out with the approval of 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, and the patients had the participation consent signed by the legal guardians.

The study used the ELISA method to measure pNF-H neurofilament levels in CSF and serum samples. Serum samples were also analyzed for creatinine levels, and motor evaluation scale results were recorded.

3. Results

Among the 42 participants, aged between 13 and 215 months, female patients were slightly predominant (55%), and most patients (74%) were diagnosed with SMA type 2. Regarding the number of SMN2 gene copies, 71% of patients had 3 copies, 22% had 2 copies, and only 7% had 4 copies. Table 1 presents the characteristics of the study group, including age, sex, SMA type, and SMN2 gene copy number.

Table 1. Characteristics of study group participants according to SMA type

	Total	SMA Type 2	SMA Type 3
Nr.	76	31	11
Sex (M)	34	16	3
Age (months) Min ÷ Max	0,67÷215	13÷215	30÷185
SMN2 copies			
2 copies	35	5	4
3 copies	38	23	7
4 copies	3	3	-

Table 2 shows the recorded parameter values 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 SMA type and SMN2 gene copy number.

Table 2. Biomarker levels and functional scale scores at the initiation of treatment

	Total	SMA Type 2	SMA Type 3
pNF-H in CSF (ng/ml) Min ÷ Max	0.035÷0.817	0.035÷0.817	0.04÷0.386
pNF-H in serum (ng/ml) Min ÷ Max	0.05÷5.268	0.137÷5.268	0.05÷1.982
Serum creatinine (mg/dl) Min ÷ Max	0.24÷0.62	0.25÷0.52	0.24÷0.62
HFMSE Min ÷ Max	0÷54	0÷34	19÷54

Before treatment initiation, the highest pNF-H neurofilament level in CSF (0.817 ng/ml) was recorded in a 16-month-old male patient, while the lowest level (0.035 ng/ml) was found in a 104-month-old male patient, both diagnosed with SMA type 2 and carrying 3 copies of the SMN2 gene.

In the serum, the highest pNF-H neurofilament level (5.268 ng/ml) was recorded in a 17-month-old male patient with SMA type 2 and 3 copies of the SMN2 gene, while the lowest level (0.05 ng/ml) was observed in a 176-month-old female patient with SMA type 3 and 2 copies of the SMN2 gene.

For creatinine, the lowest value (0.24 mg/dl) was observed in a 145-month-old female patient with SMA type 3 and 3 copies of the SMN2 gene, while the highest value (0.62 mg/dl) was recorded in a 176-month-old female patient with SMA type 3 and 2 copies of the SMN2 gene.

The highest pNF-H neurofilament levels in CSF were observed in all patients prior to treatment initiation, regardless of SMA type or SMN2 copy number. The lowest CSF pNF-H levels, likely reflecting basal neuronal metabolism, were recorded during the maintenance period, except following certain events (e.g., posture correction surgeries) before CSF collection, after which levels returned to values comparable to those of other study participants (Figure 1).

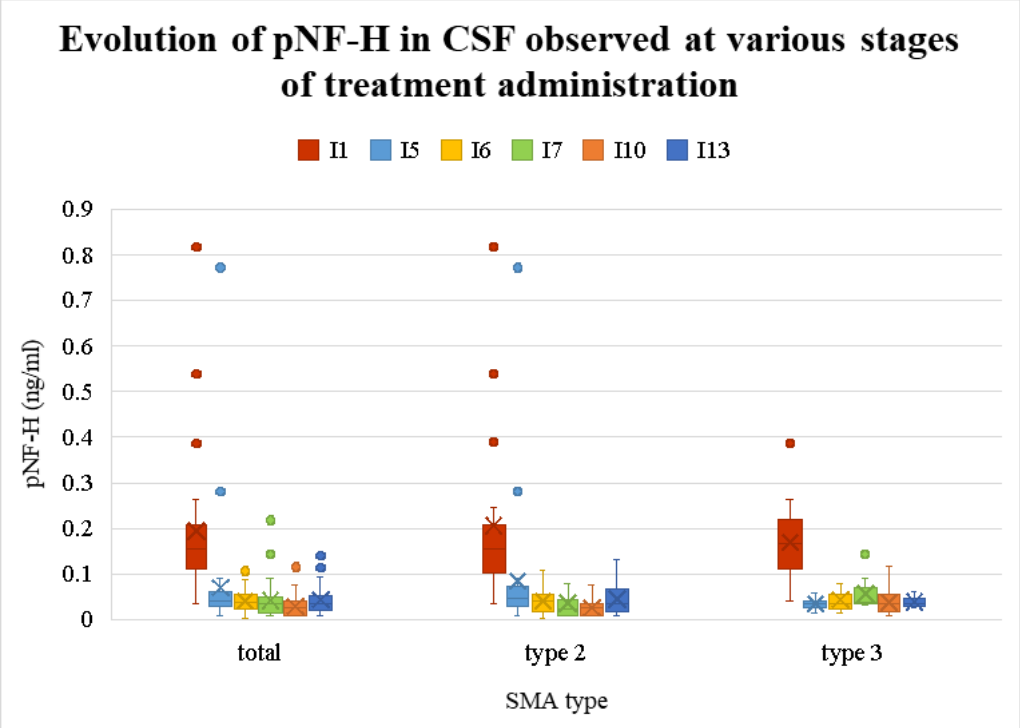

Figure 1. Evolution of pNF-H neurofilament levels in CSF at various treatment stages based on SMA type (I1: therapy initiation, I5: pre-maintenance phase, I6: 10 months post-initiation, I7: one year of therapy, I10: two years of therapy, I13: three years of therapy)

Serum pNF-H values followed the same trend as CSF, with the highest levels observed at baseline (Figure 2).

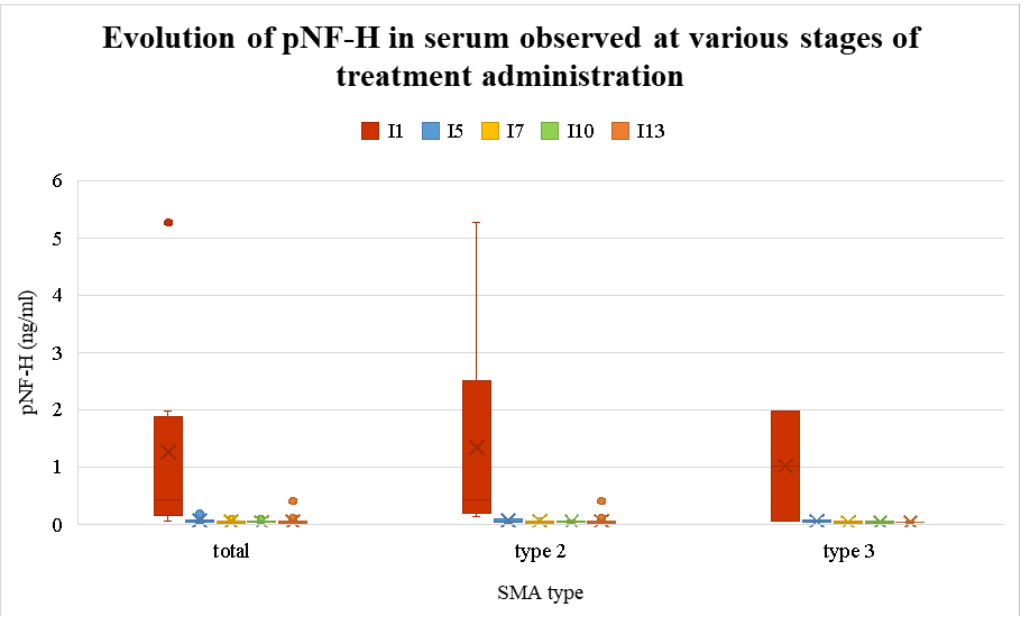

Figure 2. Evolution of pNF-H neurofilament levels in serum at various stages of treatment by SMA type (I1: therapy initiation, I5: pre-maintenance phase, I6: 10 months post-initiation, I7: one year of therapy, I10: two years of therapy, I13: three years of therapy).

Serum creatinine level increased over the three years of treatment compared to the initial values across all patient groups, although an initial decrease was observed at six months of treatment (Figure 3).

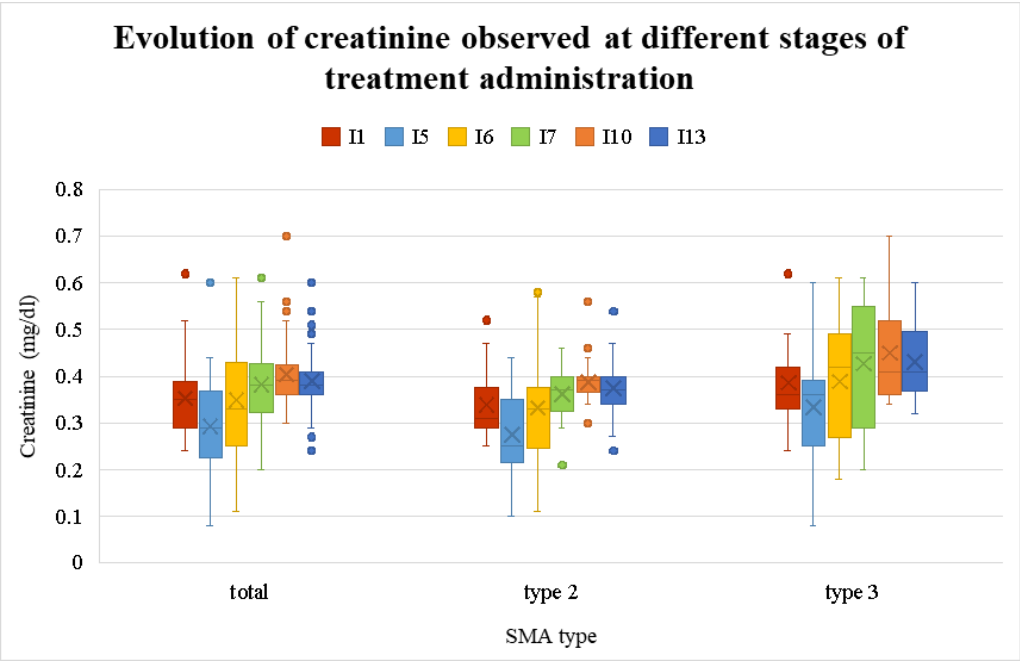

Figure 3. Evolution of serum creatinine levels at various stages of treatment by SMA type (I1: therapy initiation, I5: pre-maintenance phase, I6: 10 months post-initiation, I7: one year of therapy, I10: two years of therapy, I13: three years of therapy).

Regarding motor scale functioning, all patient groups demonstrated improvements over the three years of treatment compared to baseline, with the exception of the group with 4 copies of the SMN2 gene. This group initially showed gains in motor scale scores over the first two years, followed by a slight decline below baseline in the third year (Figure 4).

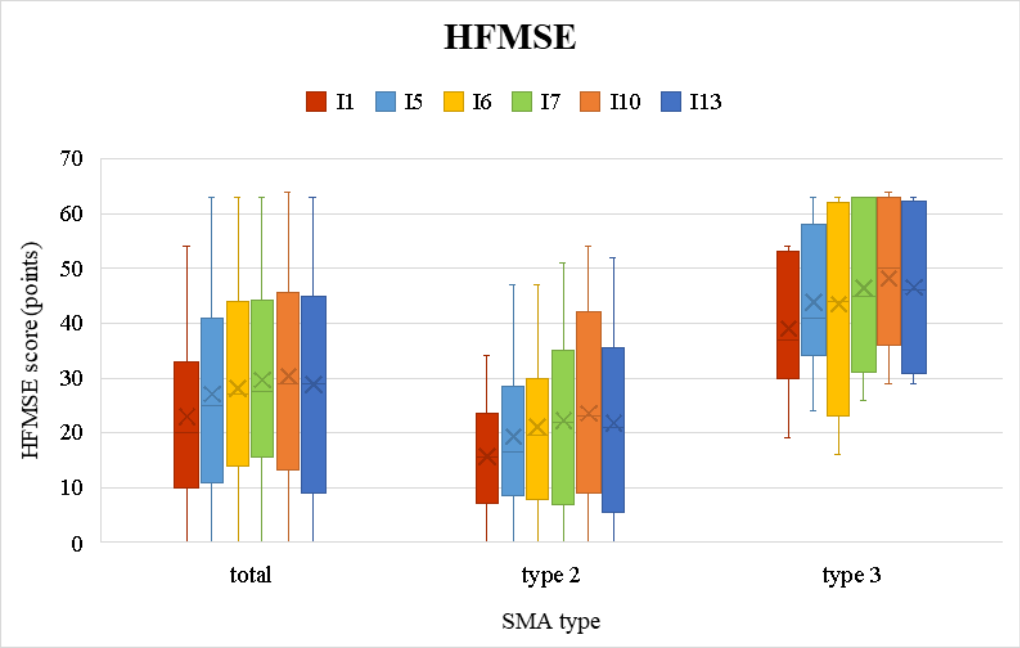

Figure 4. Evolution of motor scale performance relative to baseline across SMA types at various treatment stages (I1: therapy initiation, I5: pre-maintenance phase, I6: 10 months post-initiation, I7: one year of therapy, I10: two years of therapy, I13: three years of therapy).

Data analysis revealed several statistically significant correlations among the monitored parameters, with varying degrees of strength.

SMA type 2

In SMA type 2 patients, the number of SMN2 copies showed a weak negative correlation with pNF-H levels in CSF after one year of nusinersen treatment ($r = -0.479$) and a weak positive correlation with pNF-H levels after two years ($r = 0.440$).

Regarding the age at treatment initiation, there were statistically significant ($p < 0.01$), moderate to strong negative correlations with the HFMSE motor assessment scores across all time points ($r = -0.648$ at I1, $r = -0.718$ at I5, $r = -0.717$ at I6, $r = -0.676$ at I7, $r = -0.666$ at I10, $r = -0.691$ at I13). Age at initiation also correlated weakly negatively with serum creatinine levels at the start of maintenance treatment ($r = -0.415$) and weakly positively with CSF pNF-H levels after three years of treatment ($r = 0.403$). Additionally, age at initiation showed a moderate negative correlation with the difference in level of CSF pNF-H levels from the loading period in absolute values ($r = -0.500$), a weak negative correlation with the difference in values relative to the initial value for the loading period between I1 and I5 ($r = -0.405$), and a weak positive correlation with the change in pNF-H levels between I5 and I10, reflecting the maintenance period up to two years of treatment ($r = 0.412$).

Age at initiation was very strongly negatively correlated with the percent difference relative to baseline in serum pNF-H level after one year and after two years of treatment ($r = -0.985$ and -0.935, respectively). Differences observed between serum creatinine levels at baseline and at the beginning of the maintenance period, at one year, and at two years of treatment were weakly positively correlated with patients' age at baseline ($r = 0.421$, 0.455, and 0.435, respectively).

The performance obtained on the HFMSE scale from initiation to the time points studied was moderately and strongly negatively correlated with age at initiation (r = -0.617 at I5, -0.664 at I6, -0.673 at I7, -0.748 at I10 and -0.804 at I13).

The CSF neurofilament pNF-H level at baseline, ranging from 0.035 ng/ml for a 104-month-old patient to 0.817 ng/ml for a 16-month-old patient, was strongly positively correlated (r = 0.852) with the level of serum pNF-H from the same time point ranged from 0.137 ng/ml for a 143-month-old patient to 5.268 ng/ml for a 17-month-old patient, all with 3 copies of the SMN2 gene. The correlation with the serum pNF-H level at the beginning of the maintenance period was strongly positive (r = 0.891), the correlation with serum creatinine at two years of treatment was weakly positive (r = 0.417), but with the difference in the pNF-H level from serum at two years of treatment versus baseline and at the beginning of the maintenance period correlations of baseline CSF pNF-H level were positive and strong (r = 0.858) and very strong (r = 0.980).

The HFMSE score from baseline to the time points studied correlated moderately and weakly positively with the pNF-H value at baseline (r = 0.523 at I5, 0.431 at I6, 0.470 at I7, 0.460 at I10 and 0.528 at I13).

Serum pNF-H level at baseline was positively and strongly correlated with performance on the scales after two years of treatment (r = 0.876) and very strongly with performance after the loading period and after one year of treatment (r = 0.955 and 0.900). With scores on functional scales, baseline serum pNF-H correlated positively and strongly at I5 (r = 0.856), at I7 (r = 0.830) and at I10 (r = 0.818).

Relative to serum creatinine, serum pNF-H at baseline correlated very strongly positively at one year of treatment (r = 0.928) and very strongly negatively at two years of treatment (r = -0.935).

Baseline pNF-H serum level correlated very strongly positively with serum level at the beginning of the maintenance period (r = 0.980) and with serum creatinine at two years of treatment (r = 0.994).

Baseline serum creatinine, with values between 0.25 mg/dl in a 114-month-old patient with 4 copies of the SMN2 gene and 0.52 mg/dl in a 172-month-old patient with 3 copies of the SMN2 gene, was moderately correlated and strongly positive with the difference in the level of pNF-H in the CSF at the analyzed moments of the maintenance period compared to the level at the beginning of this period (r = 0.603 at I6, 0.532 at I7, 0.614 at I10, 0.763 at I13) and weakly negative with the difference in the level from the loading period (r = -0.457). Baseline serum creatinine correlated weakly positively with the level obtained at one year of treatment (r = 0.498).

Baseline HFMSE score, ranging from 0 points for four patients – one with 2 copies of the SMN2 gene and 76 months at baseline and the others with 3 copies of the SMN2 gene and age at baseline of 164, 177, and 215 months, and 34 points – for a patient with 3 copies of SMN2 gene and 13 months at initiation. The initial HFMSE score correlated very strongly positively with that at the beginning of the maintenance period – at I5 (r = 0.950), from I6 (r = 0.955), I7 (r = 0.957) and I10 (r = 0.907) and strongly positively with that at I13 (r = 0.860), it correlated weakly positively with the difference in CSF pNF-H from the loading period (r = 0.427) and weakly or moderately positively with the serum creatinine level after one and two years of treatment (r = 0.449 and 0.655).

The CSF pNF-H level at the start of maintenance period, with values between 0.01 ng/ml for a 23-month-old patient at initiation and 0.772 ng/ml in a 172-month-old patient, both with 3 copies of the SMN2 gene, was moderately positively correlated with the difference in serum creatinine level from baseline between all analyzed time points (r = 0.641 for I5, 0.644 for I6, 0.656 for I7, 0.651 for I10 and 0.545 for I13).

Serum creatinine at the start of maintenance period, with values of 0.1 mg/dL for a patient aged 177 months at initiation and 3 copies of the SMN2 gene, and 0.44 mg/dL for the patient with 3 copies of the SMN2 gene, 23 months at initiation and the lowest value of serum pNF-H level at I5, correlated weakly positively with serum

creatinine level at one year (r = 0.398), at two years of treatment (r = 0.453) and with performance on the motor scales from the start of treatment to the studied time points in the first two years of treatment (r = 0.467 at I5, 0.436 at I6, 0.451 at I7 and 0.489 at I10).

The score on the HFMSE scale at the beginning of the maintenance period (at 6 months of treatment), with a minimum of 0 points for three of the 5 patients with the same score at initiation and a maximum of 47 points – patient with 2 SMN2 copies and 17 months at initiation, it was strongly positively correlated with the performance obtained on the motor scales from the beginning of the treatment to each of the moments studied (r = 0.805 for I5, 0.857 for I6, 0.900 for I7, 0.826 for I10 and 0.809 for I13), very strongly positive with the value of the scores obtained (r = 0.950 with the score from I1, 0.987 to I6, 0.983 to I7, 0.952 to I10 and 0.931 to I13), weakly positive with creatinine one year after initiation (r = 0.477) and strongly positive with the level serum creatinine two years after initiation (r = 0.717).

At I6 injection, the second maintenance dose, the CSF pNF-H level was between 0.004 ng/ml for a 37-month-old patient at baseline and 0.107 ng/ml in a 131-month-old patient at baseline, both with 2 SMN2 copies and was weakly negatively correlated with the score on the functional motor scales (r = – 0.408) and with the difference in pNF-H level in the CSF during the loading period (r = -0.466). The level of serum creatinine at I6 correlated moderately positively with the HFMSE score at I6 (r = 0.540), weakly positively with that at one year – I7 (r = 0.496) and two years of treatment – I10 (r = 0.457) and with the yield engine obtained up to this point (r = 0.415).

The HFMSE score at I6 ranged between a minimum of 0 points in 2 of the three patients with the same score at loading period and a maximum of 47 points in one patient aged 23 months at baseline, and correlated very strongly positively with the other scores from the analyzed moments (r = 0.982 for I7, 0.946 for I10 and 0.914 for I13), moderately positive with creatinine at one year and two years of treatment (r = 0.521 and 0.663) and with the variation of pNF-H level in CSF from the period of load (r = 0.511), strongly positive with the variation of serum pNF-H level, relative to the initial value, from the same period (r = 0.883) and weakly negative with the difference in creatinine level up to I6 compared to the initial value (r = - 0.453).

At one year of treatment, the CSF pNF-H level ranged between 0.015 ng/ml for a patient with 3 SMN2 copies and 215 months at baseline and 0.218 ng/ml in a 131-month-old patient with 2 SMN2 copies and it was moderately negatively correlated with the change in CSF pNF-H level at 6 months and three years of treatment (r = -0.601 and -0.630).

Serum creatinine level at one year of treatment, ranging between 0.21 mg/dl in a 127-month-old patient at baseline and 0.46 mg/dl in a 23-month-old patient at baseline, both with 3 SMN2 copies, correlated weakly positive with the score on the motor scales at one year and at two years of treatment (r = 0.459 respectively 0.461), strongly negative with the evolution of the pNF-H level in the maintenance period of the first year of treatment (r = -0.883) and weak or moderately positive with the yields obtained in the first two years of treatment at the analyzed times (r = 0.408 at I5, 0.549 at I6, 0.444 at I7 and 0.458 at I10).

The HFMSE score after one year of treatment was strongly and very strongly positively correlated with the yields obtained up to each of the analyzed moments (r = 0.741 at I5, 0.820 at I6, 0.914 at I7, 0.846 at I10 and 0.809 at I13). The HFMSE score at I7 correlated moderately and weakly positively with the variation of the pNF-H level in the CSF for the loading period (r = 0.530) and for the three years of treatment (r = 0.467), very strongly positively with the HFMSE score at two years of treatment (r = 0.973) and strongly positive with the serum creatinine level (r = 0.706) and with the evolution of the serum pNF-H level compared to the initial value at two years of treatment (r = 0.823) and weakly negative with the evolution of the serum creatinine level in the two years of treatment (r = -0.406).

After two years of treatment, the CSF pNF-H level averaged 0.026 ng/ml and ranged from 0.009 ng/ml for a 127-month-old patient at baseline to 0.075 ng/ml for a 77-month-old patient at initiation, both with 3 copies of the SMN2 gene. The serum pNF-H level after 2 years of treatment ranged between 0.019 ng/ml for a patient initiated at 16 months and 0.094 ng/ml for a patient initiated at 23 months, both with 3 SMN2 copies, with a mean of values of 0.039 ng/ml.

The serum creatinine level at two years of treatment correlated moderately and weakly positively with the HFMSE score at two and three years (r = 0.562 and 0.407 respectively) and moderately and strongly positively with the returns on the motor scales obtained up to the analyzed moments (0.662 for I5, 0.556 for I6, 0.725 for I7, 0.713 for I10 and 0.632 for I13). Compared to the level of neurofilaments, serum creatinine at I10 correlated weakly positively with the evolution of the pNF-H level in the CSF during the loading period (r = 0.477) and between I1 and I6 (r = 0.465), and moderately negatively with the percentage of pNF-H variation from CSF in the maintenance period until I10, relative to the initial value (r = -0.576). Serum creatinine correlated very strongly positively with the evolution of serum pNF-H level compared to the initial value, at 6 months (r= 0.998), one year (r = 0.993) and two years of treatment (r = 0.993).

The HFMSE score at two years of treatment, with a mean of 23.55 points, a minimum of 0 points for the same two patients as at one year, and a maximum of 54 points for a patient initiated at 17 months with 3 SMN2 copies, was strongly and very strongly positively correlated with the motor yields obtained at all times studied (r = 0.741 at I5, 0.797 at I6, 0.912 at I7, 0.918 at I10 and 0.872 at I13), very strongly positively with the HFMSE score at three years of treatment (r = 0.973) and weakly negative with the variation of serum creatinine level compared to the initial value at the second dose of maintenance treatment – I6 (r = -0.423), at one year (r = -0.424) and two years of treatment (r = -0.441). Serum creatinine at two years of treatment correlated strongly positively with the change in serum pNF-H level over the two years of treatment (r = 0.813) and moderately or weakly positively with the change in CSF pNF-H level from baseline at I5 (r = 0.571), I6 (r = 0.446), I7 (r = 0.413) and I13 (r – 0.462).

At three years of treatment, the CSF pNF-H level averaged 0.045 ng/ml, ranging between 0.009 ng/ml in a patient initiated at 37 months with 2 SMN2 copies and 0.141 ng/ml in a patient initiated at 99 months with 3 SMN2 copies and correlated weakly negatively with the change in CSF pNF-H level during the loading period – in absolute values (r = -0.461) and moderately negatively with the change relative to baseline (r = -0.615). The serum level of pNF-H at three years of treatment had a mean value of 0.053 ng/ml and ranged between 0.009 ng/ml for a patient on treatment initiated at 127 months with 3 SMN2 children and 0.408 ng/ml for a patient initiated at 77 months, with 4 SMN2 copies.

The score obtained on the HFMSE scale after three years of treatment, with an average value of 21.82 points, a minimum of 0 points in the 2 patients who did not achieve an evolution on this scale and a maximum of 52 points for two patients with 3 SMN2 copies with treatment initiated at 23 and 27 months, moderately, strongly and very strongly positively correlated with the yields obtained in the analyzed time periods (r = 0.688 at I5, 0.765 at I6, 0.860 at I7, 0.868 at I10 and 0.904 at I13), weakly negative with the evolution of the serum creatinine level in the first year of treatment (r = -0.491) and weakly or moderately positive with the variation of the pNF-H level in the CSF during the loading period, in absolute value (r = 0.575) or relative to initial value (r = 0.483).

Changes in CSF pNF-H level from baseline were positively and weakly or moderately correlated for each studied time point in the maintenance period (I5, I6, I7, I10, and I13) with all baseline returns for each of these moments, with correlation coefficients ranging from 0.410 to 0.631.

The variation in CSF pNF-H level during the initiation period (I1-I5) was moderately or weakly negatively correlated with the variation in CSF pNF-H level from

the beginning of the maintenance period to each of the studied time points in this period (r = -0.515 vs. I6 - I5, 0.472 vs. I7 - I5, -0.546 vs. I10 - I5 and -0.525 vs. I13 - I5). The variation of the pNF-H level in the CSF during the initiation period (I1-I5) correlated very strongly positively with the variation of the pNF-H level in the serum during the first year of treatment – between I1 and I7 (r = 0.909), strongly for the first two years of treatment – between I1 and I10 (r = 0.890), very strong for the variation from the maintenance period of the first year of treatment – between I5 and I7 (r = 0.901) and from the beginning of the maintenance period to two years of treatment – between I5 and I10 (r = 0.983).

Variations of pNF-H level in CSF up to different time periods studied from the first two years of maintenance treatment (I6 - I1, I7 - I1 and I10 - I1) correlated positively and strongly or very strongly (with correlation coefficients ranged between 0.866 and 0.984) with the variations of serum pNF-H levels at one year and at two years of treatment, both relative to the initial moment (I7 – I1 and I10 – I1) and with the moment of starting maintenance treatment (I7 – I5 and I10 – I5).

The change in CSF pNF-H level during the initiation period (I1-I5) correlated weakly or moderately negatively with the change in serum creatinine level from baseline for I5 (r = -0.498), I6 (r = -0.434), I7 (r = -0.574) and I10 (r = -0.690).

Variations in CSF pNF-H level up to different studied time periods from the maintenance period to the start of maintenance treatment (I6–I5, I7–I5, I10–I5 and I13–I5) were moderately negatively correlated with the variation percentage of the CSF pNF-H level during the loading period compared to the initial value (r = -0.630, -0.517, -0.668 and -0.622 respectively) and weakly, moderately or strongly positive with all variations of the serum creatinine level for the studied moments compared to the initial value (I5 – I1, I6 – I1, I7 – I1, I10 – I1 and I13 – I1) with a correlation coefficient between 0.465 and 0.723.

The percentage change in CSF pNF-H level during the loading period relative to the initial value was moderately negatively correlated with the change in serum creatinine level from the initial value for each of the studied time points (r = -0.643 for I5, -0.561 for I6, -0.614 for I7, -0.622 for I10 and -0.526 for I13) and weakly or moderately positive with performance on the motor scales at one year (r = 0.465), two years (r = 0.505) and three years of treatment (r = 0.465).

The change in serum creatinine level during the loading period was moderately or strongly negatively correlated with the change in creatinine during the maintenance period compared to the level at the start of the maintenance period (r = -0.536 for I6 – I5, -0.794 for I7 – I5, -0.663 for I10 – I5 and -0.645 for I13 – I5).

SMA type 3

In the case of SMA type 3, the number of copies of the SMN2 gene correlated moderately negatively with the scores obtained on the HFMSE scale during the first two years of treatment at the studied time points (r = -0.621 at I1, -0.614 at I5, -0.622 at I6, - 0.629 at I7 and -0.649 at I10) and with the performance on the motor scales obtained in two years of treatment (r = -0.626).

The age of the patients at initiation, with a mean of 108.36 months, a minimum of 30 months and a maximum of 185 months, both patients with 3 copies of the SMN2 gene, was strongly negatively correlated with the change in serum creatinine level for the maintenance treatment period after three years of treatment (r = -0.743).

The CSF pNF-H level at treatment initiation ranged from 0.040 ng/ml – for two patients – one with 2 SMN2 gene copies and 79 months at initiation and the other with 3 SMN2 copies and 185 months at initiation, and 0, 386 ng/ml for a patient with 3 SMN2 copies and 30 months at baseline. Initial CSF pNF-H level correlated strongly positively with CSF pNF-H level at three years of treatment (r = 0.784), strongly negatively with serum creatinine level at three years of treatment (r = -0.707) and strongly positively with the variation of serum creatinine level during the three years of treatment (r = 0.736).

Initial serum creatinine level was moderately or strongly positively correlated with HFMSE scores during the first two years of treatment at the studied time points (r = 0.609 at I1, 0.721 at I5, 0.634 at I6, 0.677 at I7 and 0.643 at I10), with returns for the first and second year of treatment (r = 0.796 and 0.607 respectively) and with serum creatinine level at I6 (r = 0.781) and I10 (r = 0.861) and moderately negative with pNF-H level at the beginning of maintenance period (r = -0.636).

The initial score on the HFMSE scale, with values between 19 points for one patient with 3 SMN2 copies and age 145 months and 54 points for two patients with 2 SMN2 copies and 50 and 176 months at initiation of treatment, respectively, was strongly and very strongly positively correlated with the scores obtained at the studied times (r = 0.986 at I5, 0.888 at I6, 0.968 at I7, 0.955 at I10 and 0.921 at I13), strongly or moderately positive with the serum creatinine level at one and two years of treatment (r = 0.829 and 0.680 respectively) and moderately positive with pNF-H level in CSF at one year of treatment (r = 0.663).

CSF neurofilament pNF=H levels at the start of the maintenance period, with values between 0.014 ng/ml in a 176-month-old patient at baseline and 0.058 in a 76-month-old patient at baseline, both with 2 SMN2 copies, correlated with the variation of their level in the first year of the maintenance period moderately positive in absolute value (r = 0.604) and strongly positive in relative value to the value at the beginning of this period (r = 0.709).

Serum creatinine at the beginning of the maintenance period was moderately and strongly positively correlated with all yields obtained at the studied time points in the three years of treatment (r = 0.722 at I5, 0.756 at I6, 0.613 at I7, 0.749 at I10 and 0.656 at I13).

The score on the HFMSE scale at the beginning of the maintenance period correlated strongly positively with all yields obtained at the studied moments (r= 0.849 at I5, 0.720 at I6, 0.878 at I7, 0.887 at I10 and 0.836 at I13), very strongly positively with the scores at I6 (r = 0.910), I7 (r = 0.964), I10 (r = 0.976) and I13 (r = 0.963) and strongly positive with serum creatinine (r = 0.890 at I6, 0.870 at I10 and 0.701 at I13).

The serum creatinine level at the I6 injection was strongly positively correlated with the HFMSE scores and the creatinine level at the other time points of the maintenance period (r = 0.703 at I6, 0.864 at I7, 0.809 at I10 and 0.765 at I13 for the score, respectively 0.812 at I7, 0.870 at I10 and 0.684 at I13 for creatinine).

The HFMSE scale score at 8 months of treatment (I6) was strongly and very strongly positively correlated with yields in the studied periods (r = 0.785 for I5, 0.919 for I6, 0.941 for I7, 0.917 for I10 and 0.800 for I13) and with scores obtained (r = 0.952 at I7, 0.940 at I10 and 0.869 at I13).

CSF pNF-H level at one year of treatment, ranging between 0.031 ng/ml for two patients, one with treatment initiation at 79 months and 2 SMN2 copies, the other at 119 months and 3 SMN2 copies, and a maximum of 0.144 ng /ml for a 50-month-old patient at initiation and 2 SMN2 copies, was moderately or strongly positively correlated with the yields obtained up to the studied time points in the maintenance period (r = 0.613 for I6, 0.703 for I7, 0.701 for I10 and 0.674 for I13) and moderately positive with motor scale scores at one (r = 0.684), two (r = 0.669), and three years of treatment (r = 0.690). The CSF pNF-H level at one year of treatment was strongly positively correlated with that at two years of treatment (r = 0.755) and strongly negatively correlated with the variation of pNF-H level between the level at two years of treatment and the initial value (I10 –I1), relative to the initial value (r = -0.773) and between the level after two years of treatment and that at the beginning of maintenance treatment (I10 – I5) – in absolute value (r = -0.841) and in relative to I5 value (r = -0.878).

After one year of treatment, the score on the HFMSE scale was strongly and very strongly positively correlated with the yields obtained at the studied times (r = 0.778 at I5, 0.786 at I6, 0.941 at I7, 0.881 at I10 and 0.789 at I13), very strongly positive with the motor scores at two and three years of treatment (r = 0.962 and 0.910) and with the variation of the pNF-H level in the CSF during the maintenance period of the first

year of treatment (I7 – I5) – moderately negative in absolute values (r = - 684) and strongly negative in values relative to I5 (r = -0.708), strongly positive with creatinine level at two years of treatment (r = 0.833) and moderately positive with that at three years of treatment (r = 0.659).

CSF pNF-H level at two years of treatment, with values between 0.009 ng/ml for two SMN2 3-copy patients aged 105 and 145 months at initiation and 0.116 ng/ml for one SMN2 2-copy patient and 50 months at initiation, was moderately negatively correlated with the variation of pNF-H level in CSF during the maintenance period of the first year of treatment (I7 – I5) – r = -0.639.

After two years of treatment, the serum creatinine level was positively correlated from moderate to very strong with the returns obtained on the motor scales at the studied times (r = 0.927 at I5, 0.659 at I6, 0.814 at I7, 0.829 at I10 and 0.853 at I13) , strongly and very strongly positive with HFMSE scale score at two years (r = 0.831) and three years of treatment (r = 0.912), moderately positive with creatinine level at three years of treatment (r = 0.685), moderately negative (r = -0.612) with the variation of pNF-H level in CSF during the maintenance period of the first year of treatment in absolute value and strongly negative with that in relative value at I5 (r = -0.750).

The score on the HFMSE scale after two years of treatment, with values between 29 points for a patient with 3 SMN2 copies aged 145 months at initiation and 64 points for two patients with 2 SMN2 copies with treatment started at 176 and 50 months, was strongly and very strongly positively correlated with the yields obtained at the studied times (r = 0.809 at I5, 0.776 at I6, 0.889 at I7, 0.938 at I10 and 0.897 at I13), very strongly positively with the HFMSE score after three years (r = 0.986) , moderately positive with the level of serum creatinine after three years of treatment (r = 0.638) and moderately negative with the variation of the pNF-H level in the CSF during the maintenance period from the first year of treatment in absolute value (r = -0.681) and strongly negative with that in relative value to I5 (r = -0.714).

At three years of treatment, the CSF pNF-H level ranged between 0.026 ng/ml for a 119-month-old patient at baseline and 0.062 ng/ml for a 74-month-old patient at baseline, both with 3 SMN2 copies, with a mean of 0.038 ng/ml and was moderately negatively correlated with the change in CSF pNF-H level during the maintenance period (r = -0.670).

Serum pNF-H level at three years of treatment correlated strongly positively with the change in CSF pNF-H level from six months, one year and two years from baseline (r = 0.766, 0.884 and 0.898 respectively) and strongly negatively with serum creatinine value after three years of treatment (r = -0.834).

The serum creatinine level at three years of treatment was moderately or strongly negatively correlated with the change in CSF pNF-H level from six months, one year, two years and three years of treatment from baseline (r = -0.710, -0.826, -0.806 and -0.685 respectively) and strongly negative with the change in CSF pNF-H level from six months, one year and two years from baseline relative to baseline (r = -0.733, -0.871 and -0.839 respectively), moderate or strongly positive with the returns on the motor scales from the same periods (r = 0.782, 0.695 and 0.640 respectively).

The score on the HFMSE scale after three years of treatment, with values between 29 points for a patient with 3 SMN2 copies aged 145 months at initiation and 63 points for two patients one with 2 SMN2 copies with treatment started at 50 months and another with 3 SMN2 copies with treatment started at 185 months, was moderately, strongly or very strongly positively correlated with the yields obtained at the studied times (r = 0.862 at I5, 0.671 at I6, 0.823 at I7, 0.924 at I10 and 0.934 at I13).

The variation of the serum creatinine level during the three years of treatment was strongly positively correlated with the variation of the pNF-H level in the CSF during the maintenance treatment period of the first two years of treatment compared to the value at the beginning of this period (r = 0.766 at I5, 0.789 at I6, 0.740 to I7 and 0.779 to I10).

The variation of the serum creatinine level during the loading period (I5 – I1) is strongly negatively correlated with the variation of the serum creatinine level during the maintenance period compared to the value at the beginning of this period (r = -0.821 for I6 – I5, -0.703 for I7 – I5 , -0.882 for I10 – I5 and -0.770 for I13 – I5).

4. Discussion

Treatment with nusinersen stopped physical deterioration or even improved motor performance for most patients as measured by functional scales and serum creatinine values, changing the natural history of the disease the earlier treatment was initiated, consistent with other studies in the literature specialty [34–46].

The initial level of neurofilaments was higher in patients with SMA type 2 – more severe form than type 3, with fewer copies of the SMN2 gene and with a younger age of onset of symptoms [47–53].

The evolution of the pNF-H level in CSF and serum demonstrates the reduction or even arrest of neuronal destruction due to insufficient SMN protein synthesis, the pNF-H level being maintained during the maintenance period at a low value, probably due to neuronal basal metabolism [40,48,50,54].

Correlation of the variations in motor performance obtained by the patients in different periods of analyzed time (at 6 months, one year, two or three years of treatment) inversely proportional to the age at initiation and directly proportional to the initial values of pNF-H in the CSF. Variations in the level of pNF-H between different time intervals, in absolute values or relative to the value from the initiation of the treatment or from the beginning of the maintenance period, were correlated with the variations of the obtained motor outputs or with the variations of serum creatinine for certain periods of time, suggesting the possibility of using pNF-H monitoring as a predictive factor of the evolution of certain categories of patients [44,53,55,56].

SMA type 2

In the case of patients with SMA type 2, with a younger age at the time of initiation of therapy, better scores were obtained on motor assessment scales throughout treatment, a greater variation in pNF-H level both in CSF during the load relative to baseline as well as in serum at one and two years of treatment and a lower value of pNF-H in CSF at three years of treatment.

The yields obtained by patients with SMA type 2 at the analyzed time points were higher the younger the age at treatment initiation and the higher the initial level of pNF-H neurofilaments in the CSF. The initial level of increased serum pNF-H correlated with a better evolution of yields for the first two years of treatment.

At a higher serum creatinine level throughout treatment, the yield in each of the studied time points in the first two years of treatment was higher. Serum creatinine increased over time as a result of increased muscle activity following nusinersen treatment by arresting motor neuron degradation and muscle function and recovery or development of new motor acquisition.

For each change in CSF pNF-H level (between the value at the start of treatment and that at 6 months, one year, two and three years of treatment) higher yields were obtained for each studied time point (at 6 months, one year, two and three years of treatment).

Greater percent change in CSF pNF-H during the nusinersen loading period was reflected in better yields at one, two, and three years of treatment and was associated with smaller differences in serum creatinine at 6 months , one year, two and three years of treatment from the beginning, an aspect also observed for the absolute change in CSF pNF-H level during the loading period in relation to differences in serum creatinine level at 6 months, one year and two years of treatment compared to the initial value.

The greater variation in CSF pNF-H level during the maintenance period compared to the level at the beginning of this period correlated with a greater difference between the serum creatinine level at all studied time points compared to the initial

value, more effective arrest of motor neuron degradation by SMN protein deficiency during the maintenance period led to an increase in the serum creatinine level, so with a more intense muscle activity, without this aspect being reflected in the yield values obtained on the motor scales, the variation of the pNF-H level in the CSF in the maintenance period indirectly suggesting an increase in muscle activity.

A higher pNF-H neurofilament level at the end of the nusinersen loading period was associated with greater changes in serum creatinine level over the three years of treatment from baseline. Muscle activity increased more when at the end of the loading period the rate of motor neuron destruction, represented by the level of neurofilaments released into the CSF, was higher.

Variations in creatinine level were greater when the age at initiation of treatment was greater, suggesting the importance of initiating treatment in the recovery of muscle activity as early as possible, so that motor deficit and sequelae are as small as possible.

A greater variation in CSF pNF-H level at the studied time points in the first two years of maintenance treatment resulted in a greater variation in serum pNF-H level at one year and two years of treatment both compared to initial serum values, as well as those from the beginning of the maintenance period.

A greater change in CSF pNF-H level relative to baseline in the loading treatment period correlated with lower values of CSF pNF-H changes in the maintenance period compared to values at the beginning of this period. Halting the destruction of motor neurons in more severe forms of active disease – when more neurons are affected – was more effective during the loading period than during the maintenance period.

Higher baseline serum creatinine was associated with greater variation in CSF pNF-H at different stages of the maintenance period, but with less variation in CSF pNF-H during the loading period. Greater variation in CSF pNF-H level during the loading period was associated with less variation in pNF-H level during the maintenance period.

Greater variation in CSF pNF-H level during the loading period was associated with age and lower baseline serum creatinine, higher HFMSE score over the first three years of treatment, a higher value of serum creatinine at two years of treatment and a lower level of pNF-H neurofilaments in CSF at three years of treatment.

A higher HFMSE score at different treatment time points was associated with younger age at treatment initiation, higher serum creatinine levels, and higher motor scores throughout treatment, suggesting the importance of early initiation of nusinersen treatment for patients with SMA type 2.

SMA type 3

For patients with SMA type 3, the SMN2 gene copy number had influences on the HFMSE scale score at each time point studied in the first two years of treatment, with a higher score at higher SMN2 gene copy numbers.

A higher initial CSF pNF-H level was associated with a higher level of this parameter after three years of treatment, with a higher value of serum creatinine and a greater change in serum creatinine over three years of treatment.

Patients with higher initial serum creatinine level had higher scores on motor scales during the first two years of treatment and better performance at 6 months and one year of treatment.

Higher motor performance values over the three years of treatment were associated with higher serum creatinine values at the start of the maintenance period and at two years of treatment, as well as higher motor scale scores at all time points from the three years of treatment.

A higher level of pNF-H neurofilaments at one year of treatment correlated with achieving higher motor scale scores at one, two and three years of treatment for patients with SMA type 3. A smaller variation in the level of CSF pNF-H during maintenance period in the first year of treatment relative to the value at the beginning of this

period was associated with higher yields on motor scales in the first two years of treatment. The value of pNF-H neurofilaments at one year of treatment and the difference in the maintenance period relative to the initial value could suggest the evolution at two years of treatment in terms of the yields obtained.

A change in serum creatinine during the higher loading period was associated with a decrease in the change in serum creatinine during the maintenance period compared to the value at the beginning of this period, and a greater change in serum creatinine during the three years of treatment implied a greater variation in CSF pNF-H levels during the first two years of treatment compared to start value.

At three years of treatment, a higher value of serum creatinine was associated with a better yield achieved in the first two years of treatment, with a smaller variation in pNF-H at 6 months, one year and two years of treatment in both value - relative to baseline as well as in absolute value, and with better scores on the HFMSE scale over the three years of treatment.

A smaller change in pNF-H at 6 months, one year and two years of treatment was associated with a lower value of serum pNF-H neurofilaments at three years of treatment, so as fewer neurons were destroyed in the first two years, fewer neurofilaments were detected in the serum after three years of treatment.

Limitation

As with any study of rare diseases, the main impediment encountered in conducting the study was the small number of available cases.

SMA is a serious, debilitating disease that progresses over time. Therefore, any eligible patient receives the specific treatment in the shortest possible time, making the existence of an independent control group impossible. The control group consists of the patients included in the study by the values of the parameters from the beginning of the therapy.

The study's new challenges included the approval of new drugs for the treatment of SMA and the possibility of changing or supplementing the administered treatments.

4. Conclusions

The clinical benefits of nusinersen treatment, as expressed by improved scores on motor scales and increased performance compared to both the beginning of the therapy and the maintenance period, were reflected paraclinically by decreased levels of pNF-H neurofilaments in CSF and serum and increased serum creatinine.

A treatment-assisted increase in SMN protein level resulted in decreased neuronal degradation and improved motor performance, especially in the forms of the disease in which the damage was initially more severe—reflected by a higher level of pNF-H in CSF and serum.

In SMA types 2 and 3, higher initial CSF pNF-H level was associated with better outcomes over time and higher serum creatinine levels.

The smaller the decrease in pNF-H neurofilament levels in CSF over the course of treatment, the better the outcomes achieved at two and three years, with minimal variation in CSF pNF-H levels suggesting a favorable evolution of motor skills over this period. Conversely, greater fluctuations in CSF pNF-H levels during both the loading and maintenance phases were associated with improved outcomes over time in late-onset forms of SMA, as well as with greater increases in serum creatinine levels in SMA types 2 and 3.

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