CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST

DOCTORAL SCHOOL

MEDICINE FIELD

SPINAL MUSCULAR ATROPHIA – CLINICAL-BIOLOGICAL CORRELATIONS

THE ROLE OF CSF pNF-H NEUROFILAMENT ANALYSIS IN NUSINERSEN-TREATED PATIENTS

PHD THESIS SUMMARY

PhD Supervisor:

Professor, Ph.D. Daniela Adriana Ion

PhD Student:

Moroșan (Bădina) Mihaela

2024

TABLE OF CONTENTS

Introduction 8
I. GENERAL PART 12
Chapter 1. Spinal amyotrophy 13
1.1. Definition 13
1.2. Historical 13
1.3. Epidemiology 14
1.4. Etiology 14
1.5. SMN protein 15
1.6. Clinical manifestations 16
1.7. Classification 16
1.7.1. Depending on the type of SMA 17
1.7.2. Depending on the number of copies of the SMN2 gene 18
1.7.3. Depending on functional abilities/motor acquisitions 19
1.8. Diagnostic 19
1.9. Differential diagnosis 20
1.10. Treatment 21
1.10.1. Nusinersen (Spinraza) 21
1.10.2. Onasemnogene abeparvovec-xioi (Zolgensma) 22
1.10.3. Risdiplam (Evrysdy) 22
1.10.4. Combined treatments 22
1.10.5. Adjuvant therapies 23
1.11. Monitoring the evolution under treatment 24
1.12. Management of the patient with SMA 25
1.12.1. Management of respiratory function 25
1.12.2. Management of gastrointestinal function 25
1.12.3. Orthopedic management and functional motor recovery 26
1.12.4. Psychological management 26
1.13. Prophylaxis 26
Chapter 2. Motor functional scales 28
2.1. CHOP-INTEND 28
2.2. HINE-2 29

2.3. HFMSE 29
2.4. RULM 30
2.5. 6MWT 30
Chapter 3. pNF-H neurofilaments 32
3.1. Definition 32
3.2. Role 32
3.3. Subunits 32
3.4. Structure of subunits 33
3.5. pNF-H 33
Chapter 4. Serum creatinine 35
Chapter 5. Nusinersen (Spinraza) 36
II.PERSONAL CONTRIBUTION 38
Chapter 6. Working hypotesis 39
Chapter 7. General and secondary objectives 40
7.1. General objectives 40
7.2. Secondary objectives 40
Chapter 8. General research methodology 41
8.1. Characteristics of the study groups 41
8.2. Informed consent 41
8.3. Inclusion criteria 42
8.4. Exclusion criteria 43
8.5. Patient safety 43
8.6. Preliminary clinical evaluation 43
8.7. Anthropometric data 44
8.8. Establishing scores on motor function scales 44
8.9. Determination of pNF-H neurofilament level in CSF 44
8.9.1. Sample collection 44
8.9.2. Preanalytical processing 45
8.9.3. Checking the macroscopic appearance 45
8.9.4. Determination of cellularity 45
8.9.5. Determination of chlorine concentration 45
8.9.6. Determination of pNF-H neurofilament level 46
8.10. Determination of serum pNF-H and creatinine levels 46

8.10.1. Sample collection 46
8.10.2. Preanalytical processing 46
8.10.3. Checking the macroscopic appearance 47
8.10.4. Determination of pNF-H neurofilament level 47
8.10.5. Determination of serum creatinine level 47
8.11. The control group 47
8.12. Statistical analysis 47
Chapter 9. Changes in pNFH Levels in Cerebrospinal Fluid and Motor Evolution after the Loading Dose with Nusinersen in Different Types of Spinal Muscular Atrophy (Study 1) 49
9.1. Introduction 49
9.2. Hypotesis 49
9.3. Objectives 49
9.4. Material and method 50
9.5. Results 50
9.6. Discussion 57
9.7. Limitation 58
9.8. Conclusions 58
Chapter 10. 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 (Study 2) 60
10.1. Introduction 60
10.2. Hypotesis 60
10.3. Objectives 60
10.4. Material and method 61
10.5. Results 61
10.6. Discussion 71
10.7. Limitation 73
10.8. Conclusions 73
Chapter 11. Impact of Nusinersen on Neurofilament, Creatinine Levels, and Motor Function in Pediatric Spinal Muscular Atrophy Rehabilitation A Biomarker Analysis (1) (Study 3) 74
11.1. Introduction 74

11.2. Hypotesis 74
11.3. Objectives 74
11.4. Material and method 74
11.5. Results 75
11.6. Discussion 91
11.7. Limitation 92
11.8. Conclusions 93
Chapter 12. Importance of neurofilament pNF-H level in cerebrospinal fluid as a predictive factor and for monitoring the response to nusinersen treatment for spinal amyotrophy in the pediatric population at different time periods correlated with other clinico-biological biomarkers (Study 4) 95
12.1. Introduction 95
12.2. Hypotesis 95
12.3. Objectives 96
12.4. Material and method 96
12.5. Results 96
12.6. Discussion 130
12.6.1. SMA type 1 131
12.6.2. SMA type 2 132
12.6.3. SMA type 3 134
12.6.4. SMA with 2 copies of the SMN2 gene 135
12.6.5. SMA with 3 copies of the SMN2 gene 136
12.6.6. SMA with 4 copies of the SMN2 gene 138
12.7. Limitation 138
12.8. Conclusions 138
Chapter 13. Final discussions 141
Chapter 14. Future research directions 143
Chapter 15. Final conclusions and personal contributions 144
Bibliography 146

List of published scientific papers

1. Axente, M., Shelby, E-S., Mirea, A., Sporea, C., **Badina, M.**, Padure, L., Ion, D.A. (2021). Clinical features and genetics in non-5q spinal muscular atrophy caused by acid ceramidase deficiency. Journal of Medicine and Life, 14(3), 424 – 428, https://medandlife.org/wp-content/uploads/20.-jml-2021-0147.pdf (Chapter 1, page 20)
2. 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. https://doi.org/10.3390/jcm10235540, https://www.mdpi.com/2077-0383/10/23/5540/htm (Chapter 1, pages 22-23)
3. **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 2023, 59, 1244. https://doi.org/10.3390/medicina59071244, https://www.mdpi.com/1648-9144/59/7/1244 (Chapter 9, pages 49-59)
4. **Badina, M.**; Sporea, C.; Bejan, C.G.; 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 and PRM Research Journal, 2024, 15(1):643, https://bioclima.ro/Balneo643.pdf (Chapter 10, pages 60-73)
5. **Badina M.**, Sporea C., Bejan G.C., Mirea A., Ion D.A. Impact of Nusinersen on Neurofilament, Creatinine Levels, and Motor Function in Pediatric Spinal Muscular Atrophy Rehabilitation: A Biomarker Analysis. Rehabilitation Medicine. Balneo and PRM Research Journal, 2024, 15(2):681, https://doi.org/10.12680/balneo.2024.681, https://bioclima.ro/Balneo681.pdf (Chapter 11, pages 74-94)

Spinal muscular atrophy

Spinal amyotrophy (spinal muscular atrophy, SMA) is a rare neurodegenerative disease with autosomal recessive transmission, characterized by the gradual degradation of motor neurons in the anterior horns of the spinal cord. The disease evolves with progressive muscle weakness and atrophy of the skeletal muscles, along with respiratory and digestive manifestations that over time, in the absence of adequate treatment, can lead to respiratory failure, paralysis and even death in severe forms [1,2].

SMA, although considered the most common cause of mortality and morbidity in the pediatric population, is a rare genetic disease with a prevalence of 1-2 per 100,000 individuals and an incidence of 1 per 10,000 live births [3–5].

The cause of SMA is the insufficient synthesis of the protein necessary for the survival of motor neurons – the SMN protein, and is due in more than 95% of cases to a deletion or mutation in the 5q13 region, at the level of the SMN1 gene that codes for this protein [1].

Under normal conditions, 80-90% of the amount of SMN protein is produced by the SMN1 gene, the remaining 10-20% being produced by the SMN2 gene, which appeared as a result of inversion and duplication processes of the SMN1 gene during evolution [6,7]. The two genes are almost identical except for a few base pairs (7 in intron 6, 2 in intron 7, one in exon 7 and one in exon 8 – where thymine is replaced by cytosine at position 820) [8]. This change leads to the alteration of splicing of the messenger RNA, by activating it in another place and removing exon 7 from the structure of the SMN2 gene by some splicing initiator proteins [9]. As a result of missing exon 7, which occurs in approximately 90% of SMN2 transcripts, a structurally and functionally altered protein is synthesized that is shorter and rapidly degraded in cells by the ubiquitin-proteasome system [6–8].

The SMN2 gene is usually present in multiple copies located in the same chromosomal region as the SMN1 gene, and the amount of SMN protein produced depends on the number of copies of the SMN2 gene present and influences the age of onset of symptoms, the severity of clinical manifestations, and the rapidity of progression to paralysis, atrophy and organ failure [10–12].

SMA evolves with progressive muscle weakness and skeletal muscle atrophy, along with respiratory and digestive manifestations that over time, in the absence of adequate treatment, can lead to respiratory failure, paralysis and even death in severe forms. Muscle

weakness is frequently symmetrical and manifests predominantly proximally, at the level of the belts, affecting the lower limbs more than the upper ones, while deep tendon reflexes are abolished or greatly diminished [13].

The clinical picture is dominated by the consequences of limiting motor skills, with important postural deformities (mainly scoliosis and contractures) and consequences on the functioning of the respiratory and digestive systems [14].

The first clinical manifestations usually appear in childhood, during the period of neuromuscular development and motor acquisitions, leading in severe forms to severe functional disabilities that evolve with paralysis and muscle atrophy, respiratory and digestive complications and organ failure that can evolve to death.

Types of SMA

Depending on the age of onset of symptoms and the degree of development of physical abilities, SMA has been classified into 5 main types of the disease, subdivided into different subtypes according to the severity of clinical manifestations [15–17].

SMA type 0 is considered the most severe but also the rarest form of the disease, symptoms appear from intrauterine life and often patients require ventilatory support from birth and will never be able to sit up or control their head movements. There is usually only one copy of the SMN2 gene and life expectancy is less than 6 months [9,18].

In type 1 - the most common type (about 58% of pediatric SMA cases), also called Werdnig-Hoffmann disease, symptoms appear in the first 6 months of life and patients have a floppy infant appearance due to severe hypotonia, with predominantly axial muscle weakness and diminished deep tendon reflexes [19–22]. Children will not acquire the ability to walk, will not be able to maintain a sitting position without support, and sometimes will not even have control of head position [11,21]. From a genetic point of view, these patients most frequently have a single copy of the SMN2 gene for subtype 1A and 2 copies of the SMN2 gene for subtypes 1B and 1C, and without ventilatory support they die within the first 2 years of life [23,24].

In Dubowitz disease - as it is also called SMA type 2, which has a frequency of about 29% of pediatric cases, symptoms appear between 6 and 18 months of age [19,20]. Generally 2 or 3 copies of the SMN2 gene are present, patients can sit but cannot walk without support. Bone deformities, especially of the ribcage and scoliosis, contractures,

difficulties in ventilation and swallowing, inefficient cough appear in evolution. Life expectancy is longer, with these patients frequently reaching adulthood [25].

SMA type 3, also called Kugelberg-Welander disease, with a frequency of about 13% of pediatric cases, is usually diagnosed after the age of 18 months, sometimes even in adolescence, when the first symptoms begin to appear [11,18–20]. Frequently there are 3 copies (in type 3A), 4 or even more copies of the SMN2 gene (in type 3B) and it progresses with the gradual loss, in varying degrees of severity, of the skills gained up to that point, but the life expectancy is not affected by this disease [25].

In SMA type 4, symptoms appear in adulthood, usually after 30 years, and patients very rarely lose the ability to walk, possibly after the age of 50, and life expectancy is considered normal [18]. Generally, these patients have between 4 and 8 copies of the SMN2 gene that manage to make enough SMN protein [26].

Treatment

At the moment, 3 disease-modifying drugs are approved for the treatment of SMA due to mutations or deletions of the SMN1 gene in the q11.2-q13.3 region of chromosome 5. All these drugs work to increase the amount of SMN protein in motor neurons, but differ in mode of action, mode of administration and level of bioavailability of tissue-delivered SMN protein [27–29].

Each of the three approved drugs - Nusinersen (Spinraza), Onasemnogene abeparvovec-xioi (Zolgensma) and Risdiplam (Evrysdy), has recommendations, inclusion criteria, exclusion criteria and possible side effects, and favorable results have been recorded in studies for each treatment scheme, in terms of increasing life expectancy and improving quality of life [30–33].

Long-term studies will clarify different aspects of the recommended treatment depending on the age of the patient, the severity of the disease, the time elapsed from the onset of symptoms to the possibility of starting treatment, and the effectiveness of switching between different treatment regimens or of the simultaneous administration of more many types of drugs [6,29,30,34–36].

The results obtained following the administered treatments depend primarily on the patient's neurological status at the time of initiation of therapy, the degree of bone deformation and muscle degradation and the number of copies of the SMN2 gene present,

so that the initiation of treatment in the presymptomatic period or as soon as possible from the onset of symptoms is crucial [37–39].

For the most severe forms of SMA, with symptomatic onset before birth and a minimal chance of survival for several months, treatment initiated in utero may represent a chance for these children, according to mouse studies, potentially increasing the effectiveness of current therapies even in other forms of disease [39–41].

Adjuvant therapies

By administering drugs, the cause of the disease is treated, bringing the necessary supply of SMN protein to stop the degradation of motor neurons, but to obtain the best results for motor, respiratory and digestive functions, it is necessary to treat the effects of the gradual degradation of motor neurons, especially by recovery and toning of atrophied muscles, prevention of infections and supervision of diet [42–44].

Correct posture, positioning with orthotics, uprighting, active or passive exercise, and even surgical treatment are used to manage musculoskeletal deformities, improve lung function, and digestive symptoms [42,45].

Monitoring the evolution under treatment

With the advent of disease-modifying treatments, there is a growing need to compare the evolution of the patients' general condition and the gains obtained from a motor point of view, depending on the type of therapy instituted, the phenotypic profile of the disease, the age of treatment initiation and the severity of symptoms, with the use of biomarkers to personalize the therapy administered in order to obtain the best possible results for each individual patient.

Circulating biomarkers – the level of available SMN protein, the level of neurofilaments (exclusively neuronal components), Tau protein (neuron-specific protein) or serum creatinine provide information on treatment response, disease progression and prognosis, with the disadvantage of metabolism and clearance over time [46–50].

While serological biomarkers are in the early stages of discovery and validation for SMA, electrophysiological biomarkers have been used over time in clinical trials of neuromuscular diseases to assess the functional status and capital of viable neurons (CMAP, MUNE, SMUP, electromyography)[51–54].

The analysis of the genetic phenotype, the changes in electrophysiological and imaging aspects, as well as the evolution of the level of some circulating markers can suggest the progression of the disease, the prognosis and the response to treatment, each category having advantages and disadvantages in the individual evaluation [55,56].

With the new discoveries in science and technology, not only the mechanisms of action and the routes of administration of the applied treatments will improve, but also the ways of evaluation for the degree of hypotonia and atrophy, as well as the functional evolution of the patients under treatment with the help of computerized images and of artificial intelligence [57].

Management of the patient with SMA

The approach to the patient with SMA is multidisciplinary, because of the many clinical aspects and their consequences on different devices and systems and especially on orthopedic, respiratory, gastrointestinal and nutritional management [58,59].

Complications arising from damage to the respiratory system due to hypoventilation due to respiratory muscle weakness, ineffective cough with accumulation of secretions and superinfections are the main causes of mortality and morbidity in SMA [59–62].

Toning the respiratory muscles and stimulating coughing to eliminate secretions through physiotherapy and kinesitherapy, postural drainage, manual or mechanical suction and assisted ventilation are just a few techniques to improve respiratory function and thus tissue and organ oxygenation [62,63].

The development of functional motor skills or the regaining of the acquisitions lost due to the disease depend both on the applied physiotherapy and kinesitherapy programs, but also on the will and possibility of the patients and their relatives to perform the recommended individual exercises [62,64].

Correct posture, mobilizing joints and reducing contractures can reduce the risk of disabling forms through equipment that ensures patients' mobility and the possibility of self-care.

If necessary, orthotics, support frames or wheelchairs, and even operations to straighten and support the spine can be used to increase the quality of life [64,65].

Motor functional scales

The assessment of motor deficit and muscle strength are used in daily practice by physiotherapists and rehabilitation doctors to determine the degree of neuromuscular impairment, the severity of the disease, the prognosis and the evolution over time of various diseases, especially in response to the applied treatments [66,67].

In SMA, different functional motor assessment scales have been created and validated based on the clinical characteristics of the disease, correlated with the severity of symptoms, the age of the patients and the type of SMA. The scales, with varying degrees of complexity, assess voluntary or reflex movements, posture, ability to walk with or without support, or resistance to exertion [68,69].

Neurofilaments

Neurofilaments (NF) are exclusively neuronal protein heteropolymers and are studied more and more in recent years as possible biomarkers for neuronal aggression, degradation and death due to their increased concentration in the CSF and hence in the blood following acute neuronal conditions, in neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Charcot-Marie-Tooth disease, frontotemporal dementia, vascular or Lewy body dementia, amyotrophic lateral sclerosis, multiple sclerosis, toxic or giant axon neuropathy or in severe burns [70–73].

Neurofilaments belong to class IV of intermediate filaments and together with microfilaments and microtubules participate in the formation of the neuronal cytoskeleton [70,71].

In addition to the structural role, neurofilaments contribute to the modulation of the nerve impulse, being also involved in increasing and maintaining the axonal diameter [74]. The activity of neurofilaments is modulated by various oxidations, glycosylations, phosphorylations, nitrations, ubiquitinations, polymerizations, assemblies and disassembly - physical and biochemical processes to which they are subjected during their transport, from the site of synthesis located in the neuronal body to the axonal level where they perform the main activity [74–76].

Based on physical, chemical and recently genetic characteristics, neurofilaments have been differentiated into 5 subunits. Three of them, present both centrally and peripherally, are named according to their molecular weight - low (NF-L), medium (NF-M) and heavy (NF-H). A fourth subunit is added to them, α-internexin – in the central

nervous system, respectively peripherin – in the peripheral nervous system [70,74,77–79].

pNF-H

pNF-H results from the phosphorylation of the largest subunit of neurofilaments (NF-H), a process that gives it a high degree of stability against the various surrounding proteolytic enzymes [74,76,80]. It is ubiquitous in neurons, with a maximum concentration at the axonal level [70,81].

In diseases that affect neuronal integrity, their structural components are released and this fact implicitly leads to the appearance of the pNF-H subunit in the interneuronal space from where it reaches the CSF and then the serum [70,72,82].

The degree of damage to the neurons is directly proportional to the amount of pNF-H and other structural elements released in the CSF, an amount that has been correlated in various clinical studies with the diagnosis, the evolution of the disease and the effectiveness of the treatment administered [75,83].

The change in the level of neurofilaments depends not only on the cause that determined the degradation and destruction of neurons, but also on the degree of disease progression, the severity of symptoms and the time elapsed from the onset of symptoms to the initiation of specific treatment [71,75,83,84].

With the advent of disease-modifying treatments, the need to find biomarkers to increase the accuracy of the prognosis, follow the evolution, monitor the response to treatment and compare the effects of the different treatments administered, and for neurodegenerative diseases, neurofilaments have been increasingly often taken into account, being exclusively neuronal structural components, which appear in small quantities in the CSF as a result of the normal metabolism of the neuronal cell, but which are released in quantities corresponding to the size of the damage in case of neuronal injury or death [71,84].

Increased stability and high axonal abundance are two particularly important aspects in choosing neurofilament pNF-H subunit level to analyze as a biomarker of neuronal damage [76,80].

Serum creatinine

Serum creatinine is considered a marker of muscle activity in various neuromuscular diseases, its level being in direct correlation with the mass of muscle tissue existing in the body and with muscle metabolism (for example in Duchenne or Becker muscular dystrophy, in Kennedy's disease or in other diseases that develops with muscle atrophy regardless of etiology)[85–87].

Serum creatinine is not influenced by diet, circadian rhythm, physical effort or other biological constants, being directly correlated with muscle metabolism.

Serum creatinine is a more useful parameter in assessing the degree of muscle utilization than creatine kinase, which correlates better with the degree of muscle breakdown [88].

In neurodegenerative diseases, a decrease in the level of serum creatinine suggests the progression of the degree of muscle denervation, and the degree of decrease of this parameter correlates with the severity of the condition [89,90].

Different studies in patients with SMA have looked at creatinine level as a biomarker for the decrease in muscle activity through the progressive denervation that occurs during the natural course of the disease and the changes in serum level that occur during the introduction of specific disease-modifying therapy [11,47,48,91].

Nusinersen (Spinraza)

Nusinersen (Spinraza) is the first drug approved for the etiological treatment of SMA 5q. The active substance is an antisense oligonucleotide that prevents premature splicing of the messenger RNA, including exon 7 in the transcription process of the SMN2 gene that codes for the SMN protein and causing the production of a protein necessary for the survival of motor neurons corresponding in length and functionality to stop neuronal degradation [92,93].

Treatment is primarily aimed at maintaining or even improving motor function and improving respiratory function and implicitly the quality of life.

The medicine is conditioned in the form of an injectable solution, for intrathecal administration, packed in 5 ml vials, containing 12 mg of active substance at a concentration of 2.4 mg/ml.

The recommended dose for each administration is 5 ml (12 mg), and the treatment scheme includes two parts: the loading period – with the administration of the drug on

days 0, 14, 28 and 63 from the initiation of therapy and the maintenance period, which involves the administration a dose of medicine once every 4 months (± 2 weeks) throughout life [94]. Before administration, it is recommended to extract 5 ml of CSF to keep the intracranial pressure constant [95].

Working hypothesis

SMA is part of the category of neurodegenerative diseases that evolve with the progressive destruction and death of motor neurons, accompanied by the related consequences: the release of neuronal structural components in the neuronal interstitial space, in the CSF and in the serum, the impairment of motor function and muscle mass.

The level of released neurofilaments – and implicitly of their pNF-H component, as the structure existing exclusively at this level, should be directly proportional to the amount of damaged neurons and thus represent a way of quantifying the degree of neuronal damage. From the interstitial space, the neurofilaments reach the CSF and from there the serum, and the level of detection depends on the amount released as well as on the metabolic processes undergone and plasma clearance.

The impairment of the motor function is observed through the evolution of the scores on the functional motor assessment scales, and the degree of atrophy of the muscle mass is reflected by the serum creatinine level.

With the introduction of disease-modifying treatment, the destruction of neurons is halted, the level of pNF-H neurofilaments in the CSF should be maintained at a basal minimum due to normal neuronal metabolism, motor performance should improve as functional recovery programs are applied, and muscle mass would increase, thus increasing the serum creatinine level in accordance with the degree of muscle activity.

Monitoring parameters for motor function (score obtained on the scales) and for the intensity of muscle activity (serum creatinine level) should evolve inversely proportional to markers of neuronal degradation (pNF-H level in CSF and serum). At baseline assessment, increases in neurofilaments should be consistent with decreases in motor scores and serum creatinine, and with nusinersen treatment, neurofilaments should decrease and motor performance and serum creatinine should improve.

General objectives

- Determination of the level of pNF-H neurofilaments in the CSF according to age, type of disease, number of copies of the SMN2 gene and stage of treatment (at initiation, during the first year of the maintenance period, respectively after one, two and three years of treatment) for the pediatric population treated with nusinersen in CNCRNC Dr. Nicolae Robănescu in order to establish reference levels for pediatric patients with SMA in Romania
- Analyzing the evolution of pNF-H neurofilament levels in CSF during different treatment periods
- Monitoring the evolution of SMA through the prism of the level of neurofilaments as a biomarker of disease progression
- Establishing the importance of the pNF-H level as a predictive factor for evaluating treatment response

Secondary objectives

- Evaluation of the patients' functional status reflected by the scores obtained on the motor functional scales in different periods of treatment
- Analyzing the evolution of pNF-H neurofilament levels in serum and serum creatinine in different treatment periods
- Establishing the correlations between the level of pNF-H neurofilaments in the CSF, the value of the scores obtained on the motor assessment scales, the level of pNF-H neurofilaments in the serum and serum creatinine
- Correlation of the values and the evolution of the pNF-H neurofilament level in the CSF for the use of this parameter as a predictive marker on the treatment results over time
- Determination of the effectiveness of the administered treatment according to the evolution of the pNF-H neurofilament level compared to the initial moment

General research methodology

During the doctoral research, groups of patients diagnosed with SMA, treated with nusinersen through the National Program for Rare Diseases - Treatment with Nusinersen for Spinal Muscular Atrophy, between October 2018 and November 2023, were analyzed in the National Clinical Center for Neuropsychomotor Recovery Dr. Nicolae Robănescu

in Bucharest - monospeciality clinical hospital, dedicated to the neuropsychomotor recovery of the child, a unique structure in Romania, at the European level, with addressability throughout the country.

All eligible patients were included in the studies under strict inclusion and exclusion criteria.

The studies were approved by the Ethics Commission of the National Clinical Center for Children's Neuropsychomotor Recovery "Dr. Nicolae Robănescu" (agreement no. 7464 of October 1, 2018).

Informed consent was obtained from the legal guardians of all patients included in the studies, according to local regulations and the World Medical Association Declaration of Helsinki, revised in 2000 in Edinburgh.

All data processing complied with GDPR regulation 697/2016.

The general criteria for inclusion in the studies were: pediatric patient, the existence of a biallelic mutation or a heterozygous mutation and deletion of the SMN1 gene in the 5q13 region confirmed by genetic analysis, the existence of at least 2 copies of the SMN2 gene, the absence of any acute condition that would was able to additionally influence the level of pNF-H neurofilaments, the admission to the national nusinersen treatment program, the administration of the treatment and the performance of clinical, paraclinical and functional evaluations before each administration with the specialized staff and equipment of CNCRNC Dr. N. Robănescu.

The main exclusion criteria were: the administration of another disease-modifying treatment, the presence of another condition known for the destruction of motor neurons with an increase in pNF-H levels in CSF and serum, the existence of additional conditions that could have influenced motor performance, non-compliance with the standard scheme of treatment with nusinersen.

The clinical evaluation before the administration of each dose of treatment was carried out by a multidisciplinary team of CNCRNC Dr. N. Robănescu. Pediatric neurologists, anesthetists and pediatricians specialized and experienced in the evaluation of patients with SMA corroborated the clinical parameters, the results of the functional parameters provided by physiotherapists specialized in the evaluation of patients with this condition and the paraclinical parameters resulting from the processing of biological samples in the accredited laboratory of CNCRNC Dr. N Robanescu.

Assessments were performed by the same individuals to reduce the risk of error in analyzes and data interpretation, and drug administration was performed after obtaining multidisciplinary team agreement.

The demographic, anthropometric, clinical and paraclinical data of the participants, which were used in the studies, were taken from the observation sheets of the patients and from the data currently recorded in the computer program of CNCRNC Dr. N. Robănescu.

Physiotherapists from CNCRNC Dr. N. Robănescu, specialized in the assessment of patients with SMA, evaluated the motor skills of the subjects included in the studies using functional scales specific to the type of SMA. The evaluations were performed by the same team of specialists to minimize the degree of subjectivity.

In the conducted studies, data obtained on the CHOP-INTEND scale were used for patients diagnosed with SMA type 1 and HFMSE for those with types 2 and 3.

The CHOP-INTEND scale has been validated for patients aged 3 months to 2 years with severe motor impairment and respiratory difficulties [96]. The scale evaluates the strength and mobility of the axial and peripheral muscles with the help of 16 requirements of varying degrees of difficulty assessed with a score from 0 to 4 points (when the requirement is fulfilled correctly), the maximum score that can be obtained on this scale for the complete fulfillment of requirements being 64 points [15,97,98]. In this way, spontaneous movements, the grip reflex, the degree of flexion and extension of the limbs, the stability of the head are appreciated [15].

The Expanded Hammersmith Functional Motor Scale (HFMSE), introduced in the evaluation of patients with SMA since 2005, is a modified Hammersmith scale for use in both type 2 and type 3 SMA, for patients with late onset of symptoms [15,99].

The scale has a maximum score of 66 points which can be obtained by correctly fulfilling 33 requirements each assessed with a maximum of 2 points [69,100].

Both the absolute values recorded and those relative to the maximum score possible on the motor scales (yield) were used to be able to compare the performances achieved by the patients.

Determination of pNF-H neurofilament level in CSF

CSF samples were collected just before each intrathecal administration of nusinersen by lumbar puncture, in a sterile container, in an amount of approximately 5

ml, corresponding to the volume of drug administered, according to the drug administration protocol.

The level of pNF-H was determined by the ELISA (the enzyme-linked immunosorbent assay) technique, according to the working instructions in the leaflet issued by the manufacturer of the reagent kit, intended for the in vitro quantitative determination of pNF-H neurofilaments in CSF or serum samples of human origin and which is part of the category of medical devices for in vitro diagnosis.

Determination of serum pNF-H and creatinine levels

Blood samples for laboratory analysis for monitoring and paraclinical evaluation of patients were collected before each dose of nusinersen.

The level of pNF-H was determined by the ELISA technique, on the same type of kit as that used to determine the level of pNF-H neurofilaments in CSF.

Serum creatinine level was determined by the improved Jaffe method, based on the reaction of creatinine with sodium picrate.

The control group

The studies conducted did not have a separate control group. The data obtained just before the start of the treatment were used as a "control group" to follow the results obtained at different moments of the treatment evaluated relative to these values.

Neurofilament pNF-H levels in CSF and serum were interpreted as absolute values and compared to values obtained just before the first dose of nusinersen to assess treatment response.

The approach allowed assessment of the natural progression of this condition prior to administration of disease-modifying treatment, providing a baseline for comparison with outcomes over time.

Changes in pNFH Levels in Cerebrospinal Fluid and Motor Evolution after the Loading Dose with Nusinersen in Different Types of Spinal Muscular Atrophy (Study 1)

The first study started from the hypothesis that the loading period with nusinersen can be considered to have the greatest influence on the level of neurofilaments in the CSF, with its marked reduction as a result of providing a sufficient amount of SMN protein,

thus eliminating the cause of the abnormal destruction of motor neurons, an aspect clinically reflected by increasing scores on the motor assessment scales.

The aim of this retrospective study was to analyze the changes in pNF-H neurofilament levels in CSF after the loading period with nusinersen, corresponding to the first 6 months of disease-modifying treatment, in different types of SMA, in patients with different phenotypes and with therapy initiated at different ages compared to baseline. Additionally, we analyzed the relationship between the results obtained on the motor assessment scales and the evolution of the pNF-H level in the CSF as an expression of the reduction of motor neuron degradation.

The study included 38 patients with a genetically confirmed diagnosis of SMA, accepted in the national program of rare diseases for treatment with nusinersen between October 2018 and July 2021 within the CNCRNC Dr. N. Robănescu - all patients who had completed 6 months since initiation nusinersen treatment. The results obtained at two time points were analyzed: before the initiation of treatment (time T0) and before the administration of the 5th dose of treatment (time T1), the dose with which the maintenance treatment begins.

The study compared the results obtained for different categories of patients, according to age, type of SMA and number of SMN2 copies, to determine the category with the best response to treatment.

The mean age according to the type of SMA was between 2.07 and 215 months, with the youngest patients being those with SMA type 1.

The values of the pNF-H level in the CSF before the initiation of treatment varied greatly between different categories of patients. The lowest value of pNF-H was 0.035 ng/ml and was obtained in a 104-month-old patient with 3 copies of the SMN2 gene diagnosed with SMA type 2, and the highest value of 3.321 ng/ml was recorded in a patient aged 3.63 months, with 2 copies of the SMN2 gene, diagnosed with SMA type 1.

The wide range of pNF-H values observed in the study is also remarkable, with the highest levels of pNF-H being obtained before initiation of treatment, in the most severe forms of the disease and in patients with the lowest scores on functional motor scales, similar to the results of other studies in the literature [32,101,102].

Observations show that treatment with nusinersen can lead to improvements in motor function and a decrease in pNF-H levels for pediatric SMA patients [103]. The effectiveness of the treatment is proven by slowing down or stopping the progression of

the disease (based on clinical symptoms) and by changes in the scores obtained by the patients on the motor scales.

The correlation between CSF pNF-H level and SMA-specific motor assessment scores during the first 6 months of treatment was negative, meaning that the decrease in pNF-H level during treatment progressed with the increase in motor functional assessment scales scores, although the correlation was statistically significant only in SMA type 2.

Considering that the dose of nusinersen administered according to the drug protocol indicated by the manufacturer compensates for the lack of the necessary SMN protein and covers the necessary for the survival of motor neurons, to compare the effectiveness of the treatment taking into account the different functional motor scales applied to the patients, the percentage variation of these parameters (pNF-H level and relative score on functional scales) was more relevant than the difference in absolute values for these parameters.

The yield of changes in the pNF-H level is represented by the percentage of the difference between the initial level of neurofilaments compared to the level obtained after 6 months of treatment, and for the motor scales it is represented by the percentage of the difference between the relative value of the score 6 months after the initiation of treatment compared to initial relative value (value relative to the maximum value of the scale).

After six months of treatment with nusinersen, motor function showed improvement for the majority of patients included in the study, as demonstrated by increased scores on motor function scales, while only a few patients showed no change in scores or a decrease in them.

The findings suggest that most patients showed a positive response to nusinersen treatment in terms of increased motor function scores and decreased CSF pNF-H levels.

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 (Study 2)

In this study, changes in the level of pNF-H neurofilaments in CSF were followed during three years of maintenance treatment with nusinersen, and these changes were analyzed compared to the values at the beginning of the treatment and at the beginning of

the maintenance period for different ages and types of SMA, and the results were evaluated in terms of the performances obtained on the motor functional scales.

The aim of this retrospective study was to analyze the changes in the level of pNF-H neurofilaments in the CSF during the first year of treatment and three years after the initiation of treatment with nusinersen, in the pediatric population with late onset of clinical manifestations - in types 2 and 3 of SMA, in patients with different phenotypes and at different ages, compared to baseline. Additionally, we analyzed the relationship between the results obtained on the motor assessment scales and the evolution of the pNF-H level in the CSF as an expression of the reduction of motor neuron degradation.

The batch of this monocentric retrospective study consisted of 18 patients diagnosed with spinal muscular atrophy and treated with nusinersen between October 2018 and July 2023 through the National Program for Rare Diseases carried out within CNCRNC Dr. Nicolae Robănescu.

All patients diagnosed with type 2 or 3 SMA with at least 13 doses of treatment administered by July 2023 were included in the study.

In the study, the level of pNF-H neurofilaments in the CSF was determined three years after the start of nusinersen treatment, at 38 months. The results were analyzed both according to age, sex, type of SMA and the number of copies of the SMN2 gene, as well as from the point of view of the evolution of the scores on the functional motor scales compared to the values obtained in the first year of treatment.

From the analysis of the obtained data, no statistically significant correlations were observed between the level of pNF-H in the CSF at the 13th injection and that at the time of initiation of treatment or with the type of SMA, the number of copies of the SMN2 gene and the gender of the patients.

Most patients showed improvements in functional scores. The dynamics of motor evolution according to the yields obtained on the evaluation scales in three years of treatment, in absolute value and relative to the moment of initiation, did not depend significantly on the sex, age or type of SMA of the patients.

The most rapid decrease in pNF-H levels was achieved in the first six months of nusinersen treatment, as was also observed in studies in adult patients with late-onset SMA (types 3 and 4) as evidence of efficacy treatment in slowing or even stopping neuronal degradation due to the existence of an insufficient amount of SMN protein necessary for the survival of motor neurons [104].

The scores obtained on the motor rating scales suggest a halt in motor function degradation or even a gradual improvement in performance over time and continued treatment.

The rate of decrease in pNF-H does not correlate with a faster improvement in motor performance at any of the time points studied (6, 10 or 38 months after initiation of treatment), the level of pNF-H in the CSF cannot be considered as predictive factor of patients' evolution, but only as a diagnostic criterion, compared to studies performed for patients with lateral spinal atrophy, in which pNF-H level had both diagnostic and prognostic value [105].

The non-significant correlation of the decrease in CSF pNF-H level with the increase in motor scores may be due to the small group of patients, the varied time interval between the onset of symptoms and the initiation of therapy, the different degrees of functional motor impairment at the initiation of treatment, and the uneven compliance of patients to perform individual physical therapy, an aspect with a significant role in the functional recovery of these patients [44].

The results obtained are similar to those described in the analyzes of the level of pNF-H in CSF from the SHINE, NURTURE, EBRACE, ENDEAR and CHERSH studies regarding the control groups (healthy population aged 0 to 20 years) and the population diagnosed with SMA (before of treatment and with or without treatment) [106].

During the initiation period in SMA types 2 and 3, the evolution of the level of neurofilaments is similar to that described in studies on adult patients, totally different from the results obtained in patients with SMA type 1, for which there is a more pronounced rate of decrease in the level of neurofilaments. pNF-H neurofilaments in CSF under nusinersen treatment relative to the value at the time of initiation of therapy. The amount of pNF-H present depends on the number of viable neurons that are affected and the rate of degradation and clearance of pNF-H from the CSF [104,106,107].

The highest pNF-H neurofilament values were observed before initiation of treatment and the mean value per patient subgroup was higher for patients with SMA type 2 compared to those with type 3, similar to data from other studies on the effects of nusinersen on the pediatric population with SMA [104,106].

On the other hand, compared to the results of other studies, patients with 2 copies of the SMN2 gene had lower mean values of pNF-H in CSF compared to those with more than 2 copies of the SMN2 gene, a fact probably also influenced by the number almost

twice as many patients with more than 2 copies of the SMN2 gene but belonging to type 2 SMA, consistent with the age of onset of symptoms, which emphasizes that the SMN2 gene cannot provide the required amount of SMN protein regardless of the number of copies existing of this gene.

The fact that the youngest patient had the highest pretreatment pNF-H value suggests a more accelerated rate of neuronal destruction in the first months of life, emphasizing the importance of neonatal screening for SMA with the advent of disease-modifying therapy .

Increased pNF-H levels have been observed both in multiple sclerosis patients during periods of disease activation and correlated with disease progression in amyotrophic lateral sclerosis. However, it could be helpful in the differential diagnosis of these diseases in patients with late-onset forms of SMA [108,109].

Monitoring pNF-H levels in SMA provides information on the effectiveness of treatment in halting neuronal degradation due to SMN protein deficiency, but may also signal the onset of an associated condition affecting motor neurons before other clinical manifestations.

During treatment with nusinersen in the pediatric population diagnosed with less severe forms of SMA (types 2 and 3), the level of pNF-H in CSF decreased significantly, especially during the loading period. After that period, pNF-H remained at a low level compared to baseline values in the absence of any external event that could have caused motor neuron damage or degradation from other causes.

The decrease in pNF-H is accompanied by the improvement of scores on motor rating scales. However, the two parameters do not correlate significantly, at least for the studied group and time points of the three years of nusinersen treatment.

The increase of the pNF-H level in the CSF in the conditions of some clinical types with the late onset of SMA in children and adolescents could be part of the diagnostic criteria regarding the acute state of motor neuron degradation and the viable capital of affected neurons.

Impact of Nusinersen on Neurofilament, Creatinine Levels, and Motor Function in Pediatric Spinal Muscular Atrophy Rehabilitation A Biomarker Analysis (1) (Study 3)

The primary objectives of this study were to analyze the evolution of functional (motor function scale scores) and paraclinical (CSF and serum pNF-H level and serum creatinine) biomarkers during treatment with nusinersen, for the pediatric population diagnosed with different types of SMA and comparing the respective results with those at the initiation of treatment and at the beginning of the loading period.

This retrospective study was carried out on a group of 69 patients treated for SMA in CNCRNC Dr. Nicolae Robănescu between November 2020 and November 2023 through the National Program for Rare Diseases - Treatment with Nusinersen.

The study included all patients for whom paired serum and CSF samples, collected before intrathecal administration of the drug, at different stages of treatment (before initiation - first dose, before the start of maintenance treatment - the fifth dose, one, two or three years after the initiation of therapy - the 7th, 10th and 13th doses of the drug, respectively).

Clinically, scores on motor rating scales improved. From a paraclinical point of view, favorable changes were observed in the levels of investigated parameters, such as serum creatinine (indicative of muscle atrophy) and pNF-H levels in CSF and serum (reflecting motor neuron damage).

The highest levels of pNF-H in CSF were observed before initiation of nusinersen treatment at all time points analyzed. This trend was consistent across different types of SMA and for patients with varying numbers of copies of the SMN2 gene [104,110–114].

Patients with SMA type 1 and those with 2 copies of the SMN2 gene showed the highest levels of pNF-H, consistent with findings in the literature on disease severity factors [104,111].

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

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. However, the group of patients with SMA type 2 recorded the highest values of this parameter, while those with SMA types 1 and 3 had similar values.

Serum creatinine, indicative of muscle atrophy, decreased during the first 6 months of nusinersen treatment but increased during maintenance treatment, particularly in patients with SMA type 2 and 3. Patients diagnosed with SMA type 1 showed fluctuations relatively minor, although the level continued to rise after three years of treatment, aligning with findings on muscle atrophy studies in previous research [48,115,116]. In addition, patients experienced increases in serum creatinine levels after 3 years of nusinersen treatment compared to baseline, specifically correlated with decreased muscle wasting and increased muscle metabolism, similar to findings in the literature [47,117].

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

Decreased levels of neurofilaments in serum and CSF demonstrate slowing or even halting of abnormal neuronal degradation. The increase in serum creatinine is due to the intensification of involuntary (eg, breathing or swallowing) and voluntary muscle activity (assessed by scores on functional motor scales) as a result of improved neuronal functionality.

The unfavorable evolution of these parameters (increased pNF-H level and decreased 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 scores 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 for its intensification by increasing the number of physical therapy sessions, the duration of the sessions or the complexity of the exercises performed.

Importance of neurofilament pNF-H level in cerebrospinal fluid as a predictive factor and for monitoring the response to nusinersen treatment for spinal amyotrophy in the pediatric population at different time periods correlated with other clinico-biological biomarkers (Study 4)

In this retrospective study, the objectives of the doctoral research are addressed for the entire group of pediatric patients diagnosed with SMA 5q treated by the National Program for Rare Diseases - Treatment with Nusinersen for Spinal Muscular Atrophy, between October 2018 and November 2023, in CNCRNC Dr. Nicolae Robănescu from Bucharest.

The study compared the changes in pNF-H neurofilament levels in CSF and serum, serum creatinine and scores on functional motor assessment scales over certain time periods, across SMA types, for different numbers of SMN2 gene copies and with initiated treatment at various ages over a period of maximum three years of treatment.

Thus, the importance of determining the level of pNF-H in the CSF in monitoring the response of patients to treatment and the use of this biomarker as a predictive factor of the evolution of patients on motor functional scales were pursued.

The final study group consisted of 76 patients, and analyzes were performed from all available CSF and serum samples for the determination by the ELISA method of pNF-H neurofilament levels, and the evolution of neurofilaments, serum creatinine and motor scale scores was followed in certain points in time and for different periods, both individually and in groups of patients, depending on the type of SMA and the number of copies of the SMN2 gene.

The time points analyzed were: T0 – before treatment initiation (first dose I1), T1 – before the start of the maintenance period (fifth dose I5, 6 months after treatment initiation), T2 – sixth treatment dose I6 , 8 months after initiation, respectively T3, T4 and T5 – one, two or three years after the initiation of therapy – doses 7 (I7), 10 (I10) and 13 (I13) of the drug respectively.

From the analysis of 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 literature [109,110,118].

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 [104,111].

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 [47,115,117].

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 and the type of SMA, so in the most severe forms of the disease and as close as possible to the onset of symptoms, when the number of viable motor neurons was greater, as was the requirement of SMN protein for their survival [104,111,113,114].

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 [119,120].

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 parameters studied (age, type of SMA, number of copies of the SMN2 gene, scores obtained on the motor scales, creatinine levels serum and pNF-H neurofilaments in the serum), especially between the variations of the values obtained in different periods of treatment 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 [120–123].

The treatment-assisted increase in SMN protein level resulted in decreased neuronal degradation and improved motor performance even more so when the damage was initially more severe, reflected by a higher level of pNF-H in CSF and serum.

In the most severe form of the disease – SMA type 1, obtaining a high level of pNF-H in the CSF at the initiation of treatment could be considered a negative predictive factor for the returns on the motor scales throughout the treatment, and obtaining high values of

pNF-H neurofilament levels in CSF throughout treatment suggests a lower yield at two years of treatment. In SMA type 2 and 3, higher baseline pNF-H in CSF was associated with better returns over time and higher serum creatinine levels.

The less pNF-H neurofilaments in CSF decreased over the course of treatment, the better returns were achieved at two and three years of treatment, with little variation over time in CSF pNF-H levels suggesting an evolution better motor skills in two and three years of treatment. Greater variation in CSF pNF-H levels during both the loading and maintenance periods was associated with greater returns over time for late-onset forms and greater increases in serum creatinine (type 2 and 3 of SMA).

Patients who recovered greatly in muscle activity during the loading period had a more modest increase in motor activity during the maintenance period.

Regardless of SMN2 gene copy number, younger age at treatment initiation was associated with more severe forms of the disease, higher CSF pNF-H values, and greater variations in neurofilaments during treatment, with greater increases in yield and creatinine level over time, the variation in pNF-H level during the loading period being directly correlated with the variation in yield at the time points analyzed, while a smaller variation in CSF pNF-H during the maintenance period resulted in yields higher on the motor scales.

The observation of higher variations in serum pNF-H level at 6 months, one year and two years of treatment was associated with a higher serum creatinine level at two years of treatment, suggesting that the variation of serum pNF-H level in the time of nusinersen maintenance treatment from baseline could be a predictive factor for the intensity of muscle activity at two years of treatment.

Limitation

The results of the studies are limited by the possibility of the existence of additional factors, independent of the underlying disease, which could interfere both with the level of pNF-H and with the performances on the functional motor scales, with a particular role attributed to the degree of neuromuscular degradation at the time of treatment initiation and the individual ability to supplement drug treatment with adjuvant treatments (kinesiotherapy). Age differences within the same type of SMA were significant, with the age of initiation of treatment dependent on the availability of the newly approved drug and not on the age of onset of symptoms or speed of diagnosis.

The methods used to assess patient response to nusinersen treatment for spinal muscular atrophy are limited by the inherent aspects of each biomarker analyzed. Furthermore, the study faces the hurdles of analyzing the evolution of a rare disease due to the small size of the patient cohort.

Assessment of motor performance relies on patient cooperation, which can be challenging, particularly in pediatric populations with fluctuating moods and health conditions. In addition, supplementing treatment with adjunctive therapies such as physical therapy introduces additional complexity. Physical therapy is essential in improving various aspects of motor function, including posture, muscle tone and strength as well as patients' mobility [44]. These aspects translate into an improved quality of life for patients and their families, emphasizing the importance of a holistic approach to SMA management.

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

Final conclusions and personal contributions

In the doctoral studies carried out in the four years, I managed to include a number of 76 patients with spinal amyotrophy treated with nusinersen, an important number given that this condition is part of the category of rare diseases, and the treatment with nusinersen was approved from 2016 to worldwide and from 2018 in Romania.

Research during the doctoral period made a major contribution to understanding the degree of damage to motor neurons due to the lack of SMN protein depending on the patient's phenotype, as well as the importance of nusinersen administration for slowing down or even stopping neuronal degradation by determining the values of the pNF-H level in the CSF according to the patient's age, the type of AMS and the number of copies of the SMN2 gene before the initiation of therapy and during the first three years of treatment.

The determination of a maximum level of pNF-H during the maintenance period, a level probably due to normal neuronal metabolism, gives the possibility of using this parameter to establish the activity stage of the disease and the size of the existing neuronal capital, particularly useful information that gives the level of pNF-H in CSF value as an

indicator of the urgency of initiating specific treatment and as a **biomarker for response monitoring to the treatment**.

Establishing correlations between the level of pNF-H neurofilaments in CSF, the value of the scores obtained on the motor assessment scales, the level of pNF-H neurofilaments in serum and serum creatinine suggest the value of pNF-H as a **biomarker of disease progression**.

One of the most important aspects resulting from the data analysis was the possibility of using the degree of variation of the pNF-H level from different periods of treatment (loading period, first year of treatment, etc.) as a **predictive factor for motor evolution** for two or three years of treatment from the point of view of the functional status – reflected in the degree of increase in the yield obtained on the motor scales and the level of serum creatinine for the assessment of the intensity of the motor activity.

Correlating the evolution of functional parameters (scores on motor scales and serum creatinine level) with pNF-H levels in CSF and serum to evaluate the effectiveness of the drug treatment scheme combined with adjuvant therapy to support respiratory and digestive functions and programs of physical therapy, physiotherapy or occupational therapy represent a special personal contribution in **personalizing the patient's treatment** to obtain the best results over time.

The studies carried out contribute significantly to the understanding of the spectrum of the disease and the response to treatment, providing new perspectives in the approach of the pediatric patient with SMA and underlining the importance of introducing the determination of the pNF-H level as a routine analysis for the evaluation of these patients.

The comparative analysis of pNF-H levels in CSF and serum and the variations from different periods correlated with the yields obtained and with the serum creatinine level creates the opportunity for **comparative assessment of the response with other types of drug treatment** and with combinations of disease-modifying treatments.

The obtained results need to be validated for larger groups of patients and verified for longer periods of time, thus opening the doors to a new era for personalized treatment in SMA. The real assessment of patients with SMA involves the correlation of all clinical data and the monitoring of any available biomarkers for a correct assessment of the progress recorded and for the dynamic personalization of therapy according to the needs and possibilities of each patient.

Bibliography

1. Mercuri, E., Sumner, C. J., Muntoni, F., Darras, B. T., & Finkel RS. Spinal muscular atrophy [Internet]. [cited 2021 May 17]. Available from: https://ghr.nlm.nih.gov/condition/spinal-muscular-atrophy#statistics
2. Li W. How do SMA-linked mutations of SMN1 lead to structural/functional deficiency of the SMA protein? PLoS One. 2017;12(6):1–13.
3. Prior TW, Snyder PJ, Rink BD, Pearl DK, Pyatt RE, Mihal DC, et al. Newborn and carrier screening for spinal muscular atrophy. Am J Med Genet Part A [Internet]. 2010;152A(7):1608–16. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ajmg.a.33474
4. Weimer LH. Neuromuscular disorders in pregnancy [Internet]. 1st ed. Vol. 172, Handbook of Clinical Neurology. Elsevier B.V.; 2020. 201–218 p. Available from: http://dx.doi.org/10.1016/B978-0-444-64240-0.00012-X
5. Verhaart IEC, Robertson A, Wilson IJ, Aartsma-Rus A, Cameron S, Jones CC, et al. Prevalence, incidence and carrier frequency of 5q–linked spinal muscular atrophy – a literature review. Orphanet J Rare Dis [Internet]. 2017;12(1):124. Available from: https://doi.org/10.1186/s13023-017-0671-8
6. Lorson CL, Hahnen E, Androphy EJ, Wirth B. A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. Proc Natl Acad Sci U S A [Internet]. 1999 May 25;96(11):6307–11. Available from: https://pnas.org/doi/full/10.1073/pnas.96.11.6307
7. Sivaramakrishnan M, McCarthy KD, Campagne S, Huber S, Meier S, Augustin A, et al. Binding to SMN2 pre-mRNA-protein complex elicits specificity for small molecule splicing modifiers. Nat Commun [Internet]. 2017 Nov 14;8(1):1476. Available from: https://www.nature.com/articles/s41467-017-01559-4
8. Hua Y, Vickers TA, Baker BF, Bennett CF, Krainer AR. Enhancement of SMN2 exon 7 inclusion by antisense oligonucleotides targeting the exon. PLoS Biol. 2007;5(4):e73.
9. Rudnik-Schöneborn S, Berg C, Zerres K, Betzler C, Grimm T, Eggermann T, et al. Genotype–phenotype studies in infantile spinal muscular atrophy (SMA) type I in Germany: implications for clinical trials and genetic counselling. Clin Genet [Internet]. 2009 Aug 24;76(2):168–78. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1399-0004.2009.01200.x

10. Harada Y, Sutomo R, Sadewa AH, Akutsu T, Takeshima Y, Wada H, et al. 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 [Internet]. 2002 Sep 1;249(9):1211–9. Available from: https://doi.org/10.1007/s00415-002-0811-4
11. Gidaro T, Servais L. Nusinersen treatment of spinal muscular atrophy: current knowledge and existing gaps. Dev Med Child Neurol [Internet]. 2019 Jan 17;61(1):19–24. Available from: https://onlinelibrary.wiley.com/doi/10.1111/dmcn.14027
12. Axente M, Mirea A, Sporea C, Pădure L, Drăgoi CM, Nicolae AC, et al. Clinical and Electrophysiological Changes in Pediatric Spinal Muscular Atrophy after 2 Years of Nusinersen Treatment. Pharmaceutics [Internet]. 2022 Sep 29;14(10):2074. Available from: https://www.mdpi.com/1999-4923/14/10/2074
13. Farrar MA, Kiernan MC. The Genetics of Spinal Muscular Atrophy: Progress and Challenges. Neurotherapeutics. 2015;12(2):290–302.
14. Tiziano FD, Tizzano EF. 25 years of the SMN genes: the Copernican revolution of spinal muscular atrophy. Acta Myol myopathies cardiomyopathies Off J Mediterr Soc Myol. 2020 Dec;39(4):336–44.
15. 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 [Internet]. 2021 Jan 1;8:2329048X211008725. Available from: http://journals.sagepub.com/doi/10.1177/2329048X211008725
16. Arnold WD, Kassar D, Kissel JT. Spinal muscular atrophy: Diagnosis and management in a new therapeutic era. Muscle Nerve [Internet]. 2015 Feb 1;51(2):157–67. Available from: https://doi.org/10.1002/mus.24497
17. Dubowitz V. Chaos in the classification of SMA: A possible resolution. Neuromuscul Disord [Internet]. 1995;5(1):3–5. Available from: https://www.sciencedirect.com/science/article/pii/096089669400075K
18. 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 [Internet]. 2015 Jul;25(7):593–602. Available from: http://dx.doi.org/10.1016/j.nmd.2015.04.009

19. Verhaart IEC, Robertson A, Wilson IJ, Aartsma-Rus A, Cameron S, Jones CC, et al. Prevalence, incidence and carrier frequency of 5q–linked spinal muscular atrophy – a literature review. Orphanet J Rare Dis [Internet]. 2017 Dec 4;12(1):124. Available from: http://ojrd.biomedcentral.com/articles/10.1186/s13023-017-0671-8
20. Lally C, Jones C, Farwell W, Reyna SP, Cook SF, Flanders WD. Indirect estimation of the prevalence of spinal muscular atrophy Type I, II, and III in the United States. Orphanet J Rare Dis [Internet]. 2017 Dec 28;12(1):1–6. Available from: https://ojrd.biomedcentral.com/articles/10.1186/s13023-017-0724-z
21. Scarciolla O, Stuppia L, De Angelis MV, Murru S, Palka C, Giuliani R, et al. Spinal muscular atrophy genotyping by gene dosage using multiple ligation-dependent probe amplification. Neurogenetics [Internet]. 2006 Nov 22;7(4):269–76. Available from: https://doi.org/10.1007/s10048-006-0051-3
22. Peredo DE, Hannibal MC. The floppy infant: evaluation of hypotonia. Pediatr Rev. 2009 Sep;30(9):e66-76.
23. Nance JR. Spinal Muscular Atrophy. Continuum (Minneap Minn). 2020;26(5):1348–68.
24. Kaufmann P, McDermott MP, Darras BT, Finkel R, Kang P, Oskoui M, et al. Observational study of spinal muscular atrophy type 2 and 3: Functional outcomes over 1 year. Arch Neurol. 2011;68(6):779–86.
25. Farrar MA, Kiernan MC. The Genetics of Spinal Muscular Atrophy: Progress and Challenges. Neurotherapeutics [Internet]. 2015;12(2):290–302. Available from: https://www.sciencedirect.com/science/article/pii/S1878747923008255
26. Willis TA. Therapeutic advances in spinal muscular atrophy. Paediatr Child Heal (United Kingdom) [Internet]. 2023;33(1):23–8. Available from: https://doi.org/10.1016/j.paed.2022.12.010
27. Mercuri, E., Sumner, C. J., Muntoni, F., Darras, B. T., & Finkel RS, Mercuri E, Sumner CJ, Muntoni F, Darras BT, Finkel RS. Spinal muscular atrophy. Nat Rev Dis Prim [Internet]. 2022 Aug 4 [cited 2021 May 17];8(1):52. Available from: https://doi.org/10.1038/s41572-022-00380-8
28. Monnette A, Chen E, Hong D, Bazzano A, Dixon S, Arnold WD, et al. Treatment preference among patients with spinal muscular atrophy (SMA): a discrete choice experiment. Orphanet J Rare Dis [Internet]. 2021 Dec 20;16(1):36. Available from:

https://ojrd.biomedcentral.com/articles/10.1186/s13023-020-01667-3

29. Mirea A, Shelby ES, Axente M, Badina M, Padure L, Leanca M, et al. Combination Therapy with Nusinersen and Onasemnogene Abeparvovec-xioi in Spinal Muscular Atrophy Type I. J Clin Med [Internet]. 2021 Nov 26;10(23):5540. Available from: https://www.mdpi.com/2077-0383/10/23/5540
30. Erdos J, Wild C. Mid- and long-term (at least 12 months) follow-up of patients with spinal muscular atrophy (SMA) treated with nusinersen, onasemnogene abeparvovec, risdiplam or combination therapies: A systematic review of real-world study data. Eur J Paediatr Neurol [Internet]. 2022;39:1–10. Available from: https://www.sciencedirect.com/science/article/pii/S1090379822000629
31. Darras BT, Masson R, Mazurkiewicz-Bełdzińska M, Rose K, Xiong H, Zanoteli E, et al. FIREFISH Part 2: 24-month Efficacy and Safety of Risdiplam in Infants with Type 1 Spinal Muscular Atrophy (SMA) (4126). Neurology. 2021;96(15_supplement):1–3.
32. Finkel RS, Mercuri E, Darras BT, Connolly AM, Kuntz NL, Kirschner J, et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. N Engl J Med. 2017;377(18):1723–32.
33. Mendell JR, Al-Zaidy SA, Lehman KJ, McColly M, Lowes LP, Alfano LN, et al. Five-Year Extension Results of the Phase 1 START Trial of Onasemnogene Abeparvovec in Spinal Muscular Atrophy. JAMA Neurol. 2021;78(7):834–41.
34. Mercuri E, Pera MC, Scoto M, Finkel R, Muntoni F. Spinal muscular atrophy — insights and challenges in the treatment era. Nat Rev Neurol [Internet]. 2020;16(12):706–15. Available from: https://doi.org/10.1038/s41582-020-00413-4
35. Bitetti I, Manna MR, Stella R, Varone A. Sequential treatment with nusinersen, Zolgensma(®) and risdiplam in a paediatric patient with spinal muscular atrophytype 1: a case report. Vol. 42, Acta myologica : myopathies and cardiomyopathies : official journal of the Mediterranean Society of Myology. Italy; 2023. p. 82–5.
36. Hahn A, Günther R, Ludolph A, Schwartz O, Trollmann R, Weydt P, et al. Short-term safety results from compassionate use of risdiplam in patients with spinal muscular atrophy in Germany. Orphanet J Rare Dis [Internet]. 2022;17(1):1–10. Available from: https://doi.org/10.1186/s13023-022-02420-8

37. Kong L, Valdivia DO, Simon CM, Hassinan CW, Delestrée N, Ramos DM, et al. Impaired prenatal motor axon development necessitates early therapeutic intervention in severe SMA. Sci Transl Med [Internet]. 2021 Jan 27;13(578):eabb6871. Available from: http://stm.sciencemag.org/content/13/578/eabb6871.abstract
38. Aragon-Gawinska K, Mouraux C, Dangouloff T, Servais L. Spinal Muscular Atrophy Treatment in Patients Identified by Newborn Screening—A Systematic Review. Genes (Basel) [Internet]. 2023;14(7). Available from: https://www.mdpi.com/2073-4425/14/7/1377
39. Baptiste C, De Vivo DC. Spinal Muscular Atrophy: A Potential Target for In Utero Therapy. Clin Obstet Gynecol [Internet]. 2021;64(4). Available from: https://journals.lww.com/clinicalobgyn/fulltext/2021/12000/spinal_muscular_atrophy__a_potential_target_for_in.20.aspx
40. Oskoui M, Dangouloff T, Servais L. Universal Newborn Screening for Spinal Muscular Atrophy. JAMA Pediatr [Internet]. 2024;178(6):520–1. Available from: https://doi.org/10.1001/jamapediatrics.2024.0489
41. Nakevska Z, Yokota T. Challenges and future perspective of antisense therapy for spinal muscular atrophy: A review. Eur J Cell Biol [Internet]. 2023;102(2):151326. Available from: https://www.sciencedirect.com/science/article/pii/S0171933523000419
42. Yi YG, Shin HI, Jang DH. Rehabilitation of spinal muscular atrophy: current consensus and future direction. J Genet Med. 2020;17(2):55–61.
43. Trenkle J, Brugman J, Peterson A, Roback K, Krosschell KJ. Filling the gaps in knowledge translation: Physical therapy recommendations for individuals with spinal muscular atrophy compared to standard of care guidelines. Neuromuscul Disord [Internet]. 2021;31(5):397–408. Available from: https://www.sciencedirect.com/science/article/pii/S0960896621000407
44. Mirea A, Leanca MC, Onose G, Sporea C, Padure L, Shelby ES, et al. Physical Therapy and Nusinersen Impact on Spinal Muscular Atrophy Rehabilitative Outcome. Front Biosci [Internet]. 2022 Jun 6;27(6):179. Available from: https://www.imrpress.com/journal/FBL/27/6/10.31083/j.fbl2706179
45. Iftikhar M, Frey J, Shohan MJ, Malek S, Mousa SA. Current and emerging therapies for Duchenne muscular dystrophy and spinal muscular atrophy.

Pharmacol Ther [Internet]. 2021;220:107719. Available from: https://www.sciencedirect.com/science/article/pii/S0163725820302503

46. Smeriglio P, Langard P, Querin G, Biferi MG. The identification of novel biomarkers is required to improve adult SMA patient stratification, diagnosis and treatment. J Pers Med. 2020;10(3):1–23.
47. Freigang M, Wurster CD, Hagenacker T, Stolte B, Weiler M, Kamm C, et al. Serum creatine kinase and creatinine in adult spinal muscular atrophy under nusinersen treatment. Ann Clin Transl Neurol [Internet]. 2021 May 31;8(5):1049–63. Available from: https://onlinelibrary.wiley.com/doi/10.1002/acn3.51340
48. Alves CRRR, Zhang R, Johnstone AJ, Garner R, Nwe PH, Siranosian JJ, et al. Serum creatinine is a biomarker of progressive denervation in spinal muscular atrophy. Neurology [Internet]. 2020 Mar 3;94(9):e921–31. Available from: https://www.neurology.org/doi/10.1212/WNL.0000000000008762
49. Rudnik-Schöneborn S, Lützenrath S, Borkowska J, Karwanska A, Hausmanowa-Petrusewicz I, Zerres K. Analysis of Creatine Kinase Activity in 504 Patients with Proximal Spinal Muscular Atrophy Types I–III from the Point of View of Progression and Severity. Eur Neurol [Internet]. 1998;39(3):154–62. Available from: https://doi.org/10.1159/000007926
50. Deutsch L, Osredkar D, Plavec J, Stres B. Spinal Muscular Atrophy after Nusinersen Therapy: Improved Physiology in Pediatric Patients with No Significant Change in Urine, Serum, and Liquor 1H-NMR Metabolomes in Comparison to an Age-Matched, Healthy Cohort. Metabolites [Internet]. 2021;11(4). Available from: https://www.mdpi.com/2218-1989/11/4/206
51. Arnold WD, Porensky PN, McGovern VL, Iyer CC, Duque S, Li X, et al. Electrophysiological Biomarkers in Spinal Muscular Atrophy: Preclinical Proof of Concept. Ann Clin Transl Neurol. 2014 Jan;1(1):34–44.
52. Swoboda KJ, Prior TW, Scott CB, McNaught TP, Wride MC, Reyna SP, et al. Natural history of denervation in SMA: Relation to age, SMN2 copy number, and function. Ann Neurol [Internet]. 2005;57(5):704–12. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ana.20473
53. Kolb SJ, Coffey CS, Yankey JW, Krosschell K, Arnold WD, Rutkove SB, et al. Natural history of infantile-onset spinal muscular atrophy. Ann Neurol [Internet]. 2017;82(6):883–91. Available from:

https://onlinelibrary.wiley.com/doi/abs/10.1002/ana.25101

54. Kolb SJ, Coffey CS, Yankey JW, Krosschell K, Arnold WD, Rutkove SB, et al. Baseline results of the NeuroNEXT spinal muscular atrophy infant biomarker study. Ann Clin Transl Neurol [Internet]. 2016;3(2):132–45. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/acn3.283

55. Kariyawasam DST, D'Silva A, Lin C, Ryan MM, Farrar MA. Biomarkers and the Development of a Personalized Medicine Approach in Spinal Muscular Atrophy. Front Neurol. 2019;10:898.

56. Pino MG, Rich KA, Kolb SJ. Update on Biomarkers in Spinal Muscular Atrophy. Biomark Insights [Internet]. 2021;16:11772719211035644. Available from: https://doi.org/10.1177/11772719211035643

57. Taleb A, Rambaud P, Diop S, Fauches R, Tomasik J, Jouen F, et al. Spinal Muscular Atrophy Hypotonia Detection Using Computer Vision and Artificial Intelligence. JAMA Pediatr [Internet]. 2024;178(5):500–2. Available from: https://doi.org/10.1001/jamapediatrics.2024.0030

58. Neil EE, Bisaccia EK. Nusinersen: A Novel Antisense Oligonucleotide for the Treatment of Spinal Muscular Atrophy. J Pediatr Pharmacol Ther [Internet]. 2019;24(3):194–203. Available from: https://doi.org/10.5863/1551-6776-24.3.194

59. Farrar MA, Park SB, Vucic S, Carey KA, Turner BJ, Gillingwater TH, et al. Emerging therapies and challenges in spinal muscular atrophy. Ann Neurol [Internet]. 2017;81(3):355–68. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ana.24864

60. Bersanini C, Khirani S, Ramirez A, Lofaso F, Aubertin G, Beydon N, et al. Nocturnal hypoxaemia and hypercapnia in children with neuromuscular disorders. Eur Respir J. 2012 May;39(5):1206–12.

61. Khirani S, Colella M, Caldarelli V, Aubertin G, Boulé M, Forin V, et al. Longitudinal course of lung function and respiratory muscle strength in spinal muscular atrophy type 2 and 3. Eur J Paediatr Neurol [Internet]. 2013;17(6):552–60. Available from: https://www.sciencedirect.com/science/article/pii/S1090379813000615

62. Dunaway S, Montes J, McDermott MP, Martens W, Neisen A, Glanzman AM, et al. Physical therapy services received by individuals with spinal muscular atrophy (SMA). J Pediatr Rehabil Med. 2016;9:35–44.

63. Chatwin M, Bush A, Simonds AK. Outcome of goal-directed non-invasive ventilation and mechanical insufflation/exsufflation in spinal muscular atrophy type I. Arch Dis Child [Internet]. 2011;96(5):426–32. Available from: https://adc.bmj.com/content/96/5/426

64. Fujak A, Raab W, Schuh A, Richter S, Forst R, Forst J. Natural course of scoliosis in proximal spinal muscular atrophy type II and IIIa: descriptive clinical study with retrospective data collection of 126 patients. BMC Musculoskelet Disord [Internet]. 2013;14(1):283. Available from: https://doi.org/10.1186/1471-2474-14-283

65. Chua K, Tan CY, Chen Z, Wong HK, Lee EH, Tay SKH, et al. Long-term Follow-up of Pulmonary Function and Scoliosis in Patients With Duchenne's Muscular Dystrophy and Spinal Muscular Atrophy. J Pediatr Orthop [Internet]. 2016;36(1). Available from: https://journals.lww.com/pedorthopaedics/fulltext/2016/01000/long_term_follow_up_of_pulmonary_function_and.13.aspx

66. Vuillerot C, Payan C, Girardot F, Fermanian J, Iwaz J, Bérard C, et al. Responsiveness of the Motor Function Measure in Neuromuscular Diseases. Arch Phys Med Rehabil [Internet]. 2012;93(12):2251-2256.e1. Available from: https://www.sciencedirect.com/science/article/pii/S0003999312004042

67. Bérard C, Payan C, Hodgkinson I, Fermanian J, The MFM Collaborative Study Group. A motor function measure scale for neuromuscular diseases. Construction and validation study. Neuromuscul Disord [Internet]. 2005;15(7):463–70. Available from: https://www.sciencedirect.com/science/article/pii/S0960896605001112

68. Ribault S, Rippert P, Jain M, Le Goff L, Genod DV, Barriere A, et al. Psychometric Characteristics of the Motor Function Measure in Neuromuscular Diseases: A Systematic Review1. J Neuromuscul Dis [Internet]. 2023 May 2;10(3):301—314. Available from: https://europepmc.org/articles/PMC10408216

69. Ramsey D, Scoto M, Mayhew A, Main M, Mazzone ES, Montes J, et al. Revised Hammersmith Scale for spinal muscular atrophy: A SMA specific clinical outcome assessment tool. Singh RN, editor. PLoS One [Internet]. 2017 Feb 21;12(2):1–19. Available from: https://dx.plos.org/10.1371/journal.pone.0172346

70. Yuan A, Rao M V., Veeranna, Nixon RA. Neurofilaments and neurofilament

proteins in health and disease. Cold Spring Harb Perspect Biol [Internet]. 2017 Apr 3;9(4):a018309. Available from: http://cshperspectives.cshlp.org/lookup/doi/10.1101/cshperspect.a018309

71. Yuan A, Nixon RA. Neurofilament Proteins as Biomarkers to Monitor Neurological Diseases and the Efficacy of Therapies. Front Neurosci [Internet]. 2021 Sep 27;15(September):689938. Available from: https://www.frontiersin.org/articles/10.3389/fnins.2021.689938/full
72. Pijnenburg YALL, Janssen JC, Schoonenboom NSMM, Petzold A, Mulder C, Stigbrand T, et al. CSF Neurofilaments in Frontotemporal Dementia Compared with Early Onset Alzheimer's Disease and Controls. Dement Geriatr Cogn Disord [Internet]. 2007;23(4):225–30. Available from: https://www.karger.com/DOI/10.1159/000099473
73. Butchbach MERR. Copy number variations in the survival motor neuron genes: Implications for spinal muscular atrophy and other neurodegenerative diseases. Front Mol Biosci. 2016;3(MAR):1–10.
74. Yuan A, Rao M V., Veeranna, Nixon RA. Neurofilaments at a glance. J Cell Sci [Internet]. 2012 Jul 15;125(14):3257–63. Available from: http://jcs.biologists.org/cgi/doi/10.1242/jcs.104729
75. Kušnierová P, Zeman D, Hradílek P, Čábal M, Zapletalová O. Neurofilament levels in patients with neurological diseases: A comparison of neurofilament light and heavy chain levels. J Clin Lab Anal. 2019;33(7):1–8.
76. Petzold A. Neurofilament phosphoforms: Surrogate markers for axonal injury, degeneration and loss. J Neurol Sci [Internet]. 2005 Jun;233(1):183–98. Available from: https://www.sciencedirect.com/science/article/pii/S0022510X05001048
77. Hoffman PN, Lasek RJ. The slow component of axonal transport. Identification of major structural polypeptides of the axon and their generality among mammalian neurons. J Cell Biol [Internet]. 1975 Aug 1;66(2):351–66. Available from: https://rupress.org/jcb/article/66/2/351/77483/The-slow-component-of-axonal-transport
78. Yuan A, Sasaki T, Kumar A, Peterhoff CM, Rao M V., Liem RK, et al. Peripherin Is a Subunit of Peripheral Nerve Neurofilaments: Implications for Differential Vulnerability of CNS and Peripheral Nervous System Axons. J Neurosci [Internet]. 2012 Jun 20;32(25):8501–8. Available from:

https://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.1081-12.2012

79. Yuan A, Rao M V., Sasaki T, Chen Y, Kumar A, Veeranna, et al. Alpha-internexin is structurally and functionally associated with the neurofilament triplet proteins in the mature CNS. J Neurosci Off J Soc Neurosci [Internet]. 2006 Sep 27;26(39):10006–19. Available from: https://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.2580-06.2006

80. Wang H, Wu M, Zhan C, Ma E, Yang M, Yang X, et al. Neurofilament proteins in axonal regeneration and neurodegenerative diseases. Neural Regen Res [Internet]. 2012 Mar 15 [cited 2023 Dec 9];7(8):620–6. Available from: https://pubmed.ncbi.nlm.nih.gov/25745454

81. Laser-Azogui A, Kornreich M, Malka-Gibor E, Beck R. Neurofilament assembly and function during neuronal development. Curr Opin Cell Biol [Internet]. 2015;32:92–101. Available from: https://www.sciencedirect.com/science/article/pii/S0955067415000058

82. Rosengren LE, Karlsson JOJE, Karlsson JOJE, Persson LI, Wikkelsø C. Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. J Neurochem [Internet]. 1996 Nov 23;67(5):2013–8. Available from: https://onlinelibrary.wiley.com/doi/10.1046/j.1471-4159.1996.67052013.x

83. Gordon BA. Neurofilaments in disease: what do we know? Curr Opin Neurobiol [Internet]. 2020;61:105–15. Available from: https://www.sciencedirect.com/science/article/pii/S0959438820300398

84. Heckler I, Venkataraman I. Phosphorylated neurofilament heavy chain: a potential diagnostic biomarker in amyotrophic lateral sclerosis. J Neurophysiol [Internet]. 2022 Feb 9;127(3):737–45. Available from: https://journals.physiology.org/doi/10.1152/jn.00398.2021

85. das Neves W, Alves CRR, de Souza Borges AP, de Castro G. Serum Creatinine as a Potential Biomarker of Skeletal Muscle Atrophy in Non-small Cell Lung Cancer Patients. Front Physiol. 2021;12(April):1–5.

86. Patel SS, Molnar MZ, Tayek JA, Ix JH, Noori N, Benner D, et al. Serum creatinine as a marker of muscle mass in chronic kidney disease: Results of a cross-sectional study and review of literature. J Cachexia Sarcopenia Muscle. 2013;4(1):19–29.

87. De Rosa S, Greco M, Rauseo M, Annetta MG. The Good, the Bad, and the Serum

Creatinine: Exploring the Effect of Muscle Mass and Nutrition. Blood Purif. 2023;52(9–10):775–85.

88. Ito D, Hashizume A, Hijikata Y, Yamada S, Iguchi Y, Iida M, et al. Elevated serum creatine kinase in the early stage of sporadic amyotrophic lateral sclerosis. J Neurol [Internet]. 2019;266(12):2952–61. Available from: https://doi.org/10.1007/s00415-019-09507-6

89. Blasi L, Sabbatini D, Fortuna A, Querin G, Martinelli I, Vianello S, et al. The value of serum creatinine as biomarker of disease progression in spinal and bulbar muscular atrophy (SBMA). Sci Rep [Internet]. 2023 Oct 12;13(1):17311. Available from: https://www.nature.com/articles/s41598-023-44419-6

90. Wang L, Chen M, He R, Sun Y, Yang J, Xiao L, et al. Serum Creatinine Distinguishes Duchenne Muscular Dystrophy from Becker Muscular Dystrophy in Patients Aged ≤3 Years: A Retrospective Study. Front Neurol [Internet]. 2017 May 8;8:196. Available from: http://journal.frontiersin.org/article/10.3389/fneur.2017.00196/full

91. Milella G, Introna A, D'Errico E, Fraddosio A, Scaglione G, Morea A, et al. Cerebrospinal Fluid and Clinical Profiles in Adult Type 2–3 Spinal Muscular Atrophy Patients Treated with Nusinersen: An 18-Month Single-Centre Experience. Clin Drug Investig [Internet]. 2021;41(9):775–84. Available from: https://doi.org/10.1007/s40261-021-01071-0

92. Corey DR. Nusinersen, an antisense oligonucleotide drug for spinal muscular atrophy. Nat Neurosci [Internet]. 2017;20(4):497–9. Available from: https://doi.org/10.1038/nn.4508

93. Chiriboga CA. Nusinersen for the treatment of spinal muscular atrophy. Expert Rev Neurother [Internet]. 2017;17(10):955–62. Available from: https://doi.org/10.1080/14737175.2017.1364159

94. Medicale SIAD, Ciulu-Costinescu F. RAPORT DE EVALUARE A TEHNOLOGIILOR MEDICALE DCI : ELOSULFASE ALFA INDICAȚIA : MUCOPOLIZAHARIDOZA TIP IVA Data depunerii dosarului. 2017;(48). Available from: https://www.anm.ro/_/EVALUARE TEHNOLOGII MEDICALE/CONTESTATII/32563_Elosulfase alfa_Vimizim.pdf

95. EMA- CHMP. Anexa I Rezumatul Caracteristicilor Produsului Jcovden. 2022;1–28.

96. Glanzman AM, McDermott MP, Montes J, Martens WB, Flickinger J, Riley S, et al. Validation of the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND). Pediatr Phys Ther [Internet]. 2011;23(4):322–6. Available from: https://journals.lww.com/pedpt/Fulltext/2011/23040/Validation_of_the_Children_s_Hospital_of.2.aspx
97. Claborn MK, Stevens DL, Walker CK, Gildon BL. Nusinersen: A Treatment for Spinal Muscular Atrophy. Ann Pharmacother. 2019;53(1):61–9.
98. Glanzman AMM, Mazzone E, Main M, Pelliccioni M, Wood J, Swoboda KJJ, et al. The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND): test development and reliability. Neuromuscul Disord [Internet]. 2010/01/13. 2010 Mar;20(3):155–61. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0960896609006981
99. Glanzman AM, O'Hagen JM, McDermott MP, Martens WB, Flickinger J, Riley S, et al. Validation of the Expanded Hammersmith Functional Motor Scale in Spinal Muscular Atrophy Type II and III. J Child Neurol [Internet]. 2011 Sep 21;26(12):1499–507. Available from: https://doi.org/10.1177/0883073811420294
100. O'Hagen JM, Glanzman AM, McDermott MP, Ryan PA, Flickinger J, Quigley J, et al. An expanded version of the Hammersmith Functional Motor Scale for SMA II and III patients. Neuromuscul Disord [Internet]. 2007;17(9):693–7. Available from: https://www.sciencedirect.com/science/article/pii/S0960896607001861
101. Darras BT, Crawford TO, Finkel RS, Mercuri E, De Vivo DC, Oskoui M, et al. Neurofilament as a potential biomarker for spinal muscular atrophy. Ann Clin Transl Neurol [Internet]. 2019 May 17 [cited 2024 May 9];6(5):932–44. Available from: https://onlinelibrary.wiley.com/doi/10.1002/acn3.779
102. Darras BT, Sumner CJ, Muntoni F, Crawford TO, Finkel RS, Mercuri E, et al. Plasma Phosphorylated Neurofilament Heavy Chain Levels Over Time in Participants With Infantile-and Later-onset SMA: Data from the SHINE Study (1692). AAN Enterprises; 2020.
103. De Wel B, De Schaepdryver M, Poesen K, Claeys KG. Biochemical and clinical biomarkers in adult SMA 3–4 patients treated with nusinersen for 22 months. Ann Clin Transl Neurol. 2022;9(8):1241–51.
104. Badina M, Bejan GC, Sporea C, Padure L, Mirea A, Leanca MC, 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 (Kaunas) [Internet]. 2023 Jul 4;59(7):1244. Available from: https://www.mdpi.com/1648-9144/59/7/1244

105. Poesen K, De Schaepdryver M, Stubendorff B, Gille B, Muckova P, Wendler S, et al. Neurofilament markers for ALS correlate with extent of upper and lower motor neuron disease. Neurology. 2017 Jun;88(24):2302–9.
106. Paris A, Bora P, Parolo S, MacCannell D, Monine M, van der Munnik N, et al. A pediatric quantitative systems pharmacology model of neurofilament trafficking in spinal muscular atrophy treated with the antisense oligonucleotide nusinersen. CPT Pharmacometrics Syst Pharmacol. 2023 Feb;12(2):196–206.
107. Paris A, Bora P, Parolo S, Monine M, Tong X, Eraly S, et al. An age-dependent mathematical model of neurofilament trafficking in healthy conditions. CPT Pharmacometrics Syst Pharmacol. 2022 Apr;11(4):447–57.
108. De Schaepdryver M, Jeromin A, Gille B, Claeys KG, Herbst V, Brix B, et al. Comparison of elevated phosphorylated neurofilament heavy chains in serum and cerebrospinal fluid of patients with amyotrophic lateral sclerosis. J Neurol Neurosurg & Psychiatry [Internet]. 2018 Apr 1;89(4):367 LP – 373. Available from: http://jnnp.bmj.com/content/89/4/367.abstract
109. Li JJY, Dai Y, Sun XXH, Ren HTH, Shen DDC, Yang XZX, et al. Comparison of neurofilament light and heavy chain in spinal muscular atrophy and amyotrophic lateral sclerosis: A pilot study. Brain Behav. 2023 May;13(5):e2997.
110. De Wel B, De Schaepdryver M, Poesen K, Claeys KG. Biochemical and clinical biomarkers in adult <scp>SMA</scp> 3–4 patients treated with nusinersen for 22 months. Ann Clin Transl Neurol [Internet]. 2022 Aug 14;9(8):1241–51. Available from: https://onlinelibrary.wiley.com/doi/10.1002/acn3.51625
111. Lapp HS, Freigang M, Hagenacker T, Weiler M, Wurster CD, Günther R. Biomarkers in 5q-associated spinal muscular atrophy—a narrative review. J Neurol [Internet]. 2023 Sep 8;270(9):4157–78. Available from: https://link.springer.com/10.1007/s00415-023-11787-y
112. Wurster CD, Günther R, Steinacker P, Dreyhaupt J, Wollinsky K, Uzelac Z, et al. Neurochemical markers in CSF of adolescent and adult SMA patients undergoing nusinersen treatment. Ther Adv Neurol Disord [Internet]. 2019 Jan

10;12:175628641984605. Available from: http://journals.sagepub.com/doi/10.1177/1756286419846058

113. Faravelli I, Meneri M, Saccomanno D, Velardo D, Abati E, Gagliardi D, et al. Nusinersen treatment and cerebrospinal fluid neurofilaments: An explorative study on Spinal Muscular Atrophy type 3 patients. J Cell Mol Med [Internet]. 2020 Mar 7;24(5):3034–9. Available from: https://onlinelibrary.wiley.com/doi/10.1111/jcmm.14939
114. Badina M, Sporea C, Bejan GC, Mirea A, Ion DA. 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 [Internet]. 2024 Mar 31;15(Vol.15, 1):643. Available from: https://bioclima.ro/Balneo643.pdf
115. Sarıkaya Uzan G, Paketçi C, Günay Ç, Edem P, Özsoy Ö, Hız Kurul S, et al. The Effect of Nusinersen Therapy on Laboratory Parameters of Patients with Spinal Muscular Atrophy. Neuropediatrics [Internet]. 2022 Oct 24;53(05):321–9. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0042-1750719
116. Stolte B, Nonnemacher M, Kizina K, Bolz S, Totzeck A, Thimm A, et al. Nusinersen treatment in adult patients with spinal muscular atrophy: a safety analysis of laboratory parameters. J Neurol [Internet]. 2021 Dec 25;268(12):4667–79. Available from: https://link.springer.com/10.1007/s00415-021-10569-8
117. Silva P. Serum Creatinine Levels Reflect Disease Severity in Muscular Dystrophy Patients [Internet]. 2015 [cited 2024 Jan 5]. Available from: https://musculardystrophynews.com/news/serum-creatinine-levels-reflect-disease-severity-in-muscular-dystrophy-patients/
118. Badina M, Sporea C, Bejan GC, Mirea A, Ion DA. Impact of Nusinersen on Neurofilament, Creatinine Levels, and Motor Function in Pediatric Spinal Muscular Atrophy Rehabilitation: A Biomarker Analysis. Creat Levels, Mot Funct Pediatr Spinal Muscular Atrophy Rehabil A Biomark Anal Rehabil Med Balneo PRM Res J. 2024;15(2):681.
119. Xing X, Liu X, Li X, Li M, Wu X, Huang X, et al. Insights into spinal muscular atrophy from molecular biomarkers. Neural Regen Res [Internet]. 9900; Available from:

https://journals.lww.com/nrronline/fulltext/9900/insights_into_spinal_muscular_atrophy_from.410.aspx

120. Li D, Yang J, Wang X, Yang L, Luo R, Huang S. Analysis of the efficacy and adverse effects of nusinersen in the treatment of children with spinal muscular atrophy in China. Brain Behav. 2024 Jul;14(7):e3630.
121. Axente M, Sporea C, Mirea A, Burcea CC, Ion DA. Time-Efficacy in SMA Type 1 and 2 Cases Treated with Nusinersen. Balneo PRM Res J [Internet]. 2023 Jun 23;14(Vol.14, 2):566. Available from: http://bioclima.ro/Balneo566.pdf
122. Chen Y. Effects of nusinersen on motor function in children with spinal muscular atrophy : a retrospective study. 2024;(July):1–8.
123. Badina M, Sporea C, Bejan GC, Mirea A, Ion DA. Impact of Nusinersen on Neurofilament , Creatinine Levels , and Motor Function in Pediatric Spinal Muscular Atrophy Rehabilitation : A Biomarker Analysis. 2024;1–23.