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# **PhD THESIS**

**CLINICAL, GENETIC, AND THERAPEUTIC CORRELATIONS  
IN PATIENTS WITH SPINAL MUSCULAR ATROPHY IN  
ROMANIA**

**- ABSTRACT -**

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# ABSTRACT

## INTRODUCTION

Spinal Muscular Atrophy (SMA) is a devastating neuromuscular condition primarily affecting the motor neurons in the spinal cord, leading to progressive muscular weakness and atrophy. SMA is classified based on the age of onset and severity of symptoms, ranging from Type I, the most severe form, to Type IV, which is a milder adult form. Despite its rarity, SMA remains the leading genetic cause of infant mortality and significant morbidity among children, with an estimated incidence of 1 in 10,000 births. Treatment for SMA has significantly advanced in the last decade with the introduction of gene therapies and splicing modifiers like nusinersen and risdiplam, which have shown the ability to significantly improve patients' quality of life. These treatments aim to increase the production of functional SMN protein, essential for the survival of motor neurons. Nusinersen, for example, was the first approved treatment and has demonstrated effectiveness in slowing disease progression even in severe forms.

The present work aimed to conduct a comprehensive examination of two cohorts of patients diagnosed with SMA by exploring clinical and genetic aspects regarding patient mobility and quality of life over extended periods. The study conducted for this thesis was structured in two parts. The first part assesses the motor function aspects of the studied patients after completing treatment with nusinersen, while the second part investigated a group of patients who were subjected to a comprehensive assessment test to reveal aspects related to quality of life after nusinersen therapy.

The doctoral thesis comprises two studies, one cross-sectional and one prospective-longitudinal, conducted in accordance with the Principles of the Declaration of Helsinki (1975, revised 2013), and with the approval of the ethics committee. The doctoral research includes the following studies:

Study 1 – Nusinersen improves motor function in Types 2 and 3 SMA over time.

Study 2 – Evaluation of the quality of life of Romanian patients with SMA under Nusinersen treatment. Published in 2024 in ISI journals (Biomedicine and Neurology International), the research offers insights to improve the management of patients with SMA, enhancing knowledge about the disease and its treatment in the scientific community.

# **PERSONAL CONTRIBUTIONS**

## **STUDY I. NUSINERSEN IMPROVES MOTOR FUNCTION IN TYPES 2 AND 3 SMA OVER TIME.**

### **Material and method**

The first study included 37 Romanian patients diagnosed with various forms of SMA, tracking their progress over 54 months, from January 2019 to June 2023, at the Neurology departments of the Fundeni Clinical Institute in Bucharest and the CF Clinical Hospital in Timisoara. The patients were treated with intrathecally administered nusinersen, starting in 2019. The research involved a detailed collection of clinical data and periodic evaluations using the Expanded Hammersmith Functional Motor Scale (HFMSE) and the Revised Upper Limb Module (RULM), both specially validated for SMA. The main goal was to assess the efficacy of nusinersen and to understand the clinical progression of the disease. Results were analyzed based on key variables such as age at symptom onset, specific type of SMA, type of exon deletion, and the number of copies of the SMN2 gene. This research aims to provide an in-depth perspective on the treatment and progression of SMA in a clinical context specific to Romania.

### **Results**

Thirty-seven Romanian patients diagnosed with SMA underwent a 54-month therapeutic regimen with nusinersen, demonstrating a multifactorial approach that included detailed anamnesis, genetic testing, and regular scoring of motor activity through RULM and HFSME. This longitudinal study aimed to track the progression of SMA and response to ongoing treatment and create a substantial dataset for future analyses.

The collected data provide a solid foundation for further analyses that could explore correlations between specific genetic variables and individual responses to treatment, thus facilitating the personalization and optimization of therapies for patients. These data are also valuable for assessing the impact of demographic factors, such as age and sex, on disease progression and treatment efficacy. Future analyses could include advanced statistical modeling and artificial intelligence to identify predictive patterns that help anticipate disease progression based on patients' initial characteristics. These analyses could contribute to developing more targeted therapeutic strategies, thereby increasing the effectiveness of medical interventions and improving the quality of life for patients with SMA.

The average age at which symptoms first appeared was 36 months, with a wide range from 12 to 72 months, reflecting significant variability in the age of onset. RULM and HFSME scores also varied, with a median RULM score of 28 and a median HFSME score of 13. Neither variable followed a normal distribution, suggesting that non-parametric statistical methods will be used for further analysis.

The distribution of patients across SMA types showed a predominance of type 3 (62.2%), suggesting that a larger proportion of patients are affected by a form of SMA with a later and less severe onset. Most patients exhibited deletions in both exons 7 and 8, indicating that the most common genetic mutation in the cohort is the deletion of both exons. Most patients have 3 copies of the SMN2 gene, a crucial factor in disease severity and response to treatment. Approximately 24.3% of patients did not complete treatment with nusinersen, indicating a significant dropout rate.

Kaplan-Meier survival analysis (Figure 1) was used to better understand dropout rates, which highlighted significant differences in time to dropout between patients with SMA type 2 and those with type 3. These differences are crucial for identifying potential barriers that may lead to premature treatment discontinuation and for optimizing strategies to keep patients in treatment.

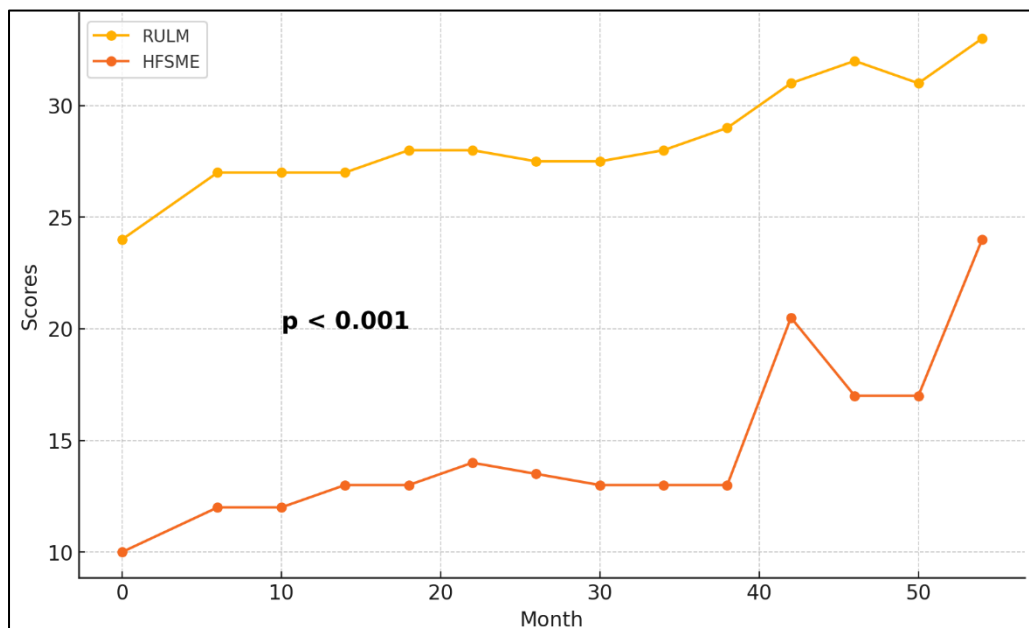


Figure 1. Graphical Representation of Paired Measurements of RULM and HFSME Scores Throughout the Study

Based on the analysis of numerical and categorical variables, valuable insights were obtained about the demographic and genetic characteristics of the patients, as well as their response to treatment. This information is crucial for personalizing treatment and improving the

management of SMA. Continuous monitoring and periodic evaluation of dropout rates are essential for proactive interventions and more effective long-term disease management, contributing to improving care standards for patients with SMA

## **STUDY II. EVALUATION OF THE QUALITY OF LIFE OF ROMANIAN PATIENTS WITH SMA UNDER NUSINERSEN TREATMENT**

### **Material and method**

The second study assessed the quality of life of 43 Romanian patients diagnosed with SMA types 1, 2, or 3, using an online questionnaire administered between August 2021 and January 2022. Initially planned as face-to-face interviews, the restrictions imposed by the COVID-19 pandemic necessitated a shift to a digital format, utilizing the Google® Forms platform for collecting responses. The study did not involve collecting personal data, thus ensuring the anonymity and confidentiality of the participants. The SF-36 questionnaire measures eight quality dimensions of life, from physical functioning and vitality to general health and bodily pain. It was initially validated for adults and has gradually been adapted for youths starting at 12. Parents or guardians provided information for younger children. The assessment also included questions about demographic and disease-specific factors, such as the type of SMA and the use of ventilatory support. Scores from the SF-36 are calculated by transforming responses into percentage scores, which are then aggregated to produce scalar scores for each dimension. Missing data from specific responses are omitted from the final calculations to maintain the accuracy of the scores. This detailed methodology and the attention given to data confidentiality and accuracy underscore the value of the SF-36 in clinical evaluations and research studies across various health contexts.

### **Results**

The second part of our study involved administering a quality-of-life questionnaire to a group of 43 patients, providing new and comprehensive data on the improvements in their performance following treatment with nusinersen. We analyzed a diverse sample of patients ranging from 3 to 72 years old, with an age distribution skewed to the right, indicating a predominance of younger individuals in our cohort. In this group, 30% are children under 14 years, and 70% are individuals over this age, with an average age of 22.52 years. This suggests that most of our patients are relatively young, also confirmed by the median age of 20 years.

Our analysis revealed a significant age variability, with an interquartile range showing a wide range of ages among patients. Additionally, the high standard deviation underscores the age diversity within the group. The Shapiro-Wilk test confirmed that the age distribution is not normal, with a significant p-value, indicating the specificity of the age distribution in our cohort (Figure 2.). This variability in age provides valuable insights into the disease's impact across different life stages and helps tailor interventions more appropriately to each age group.

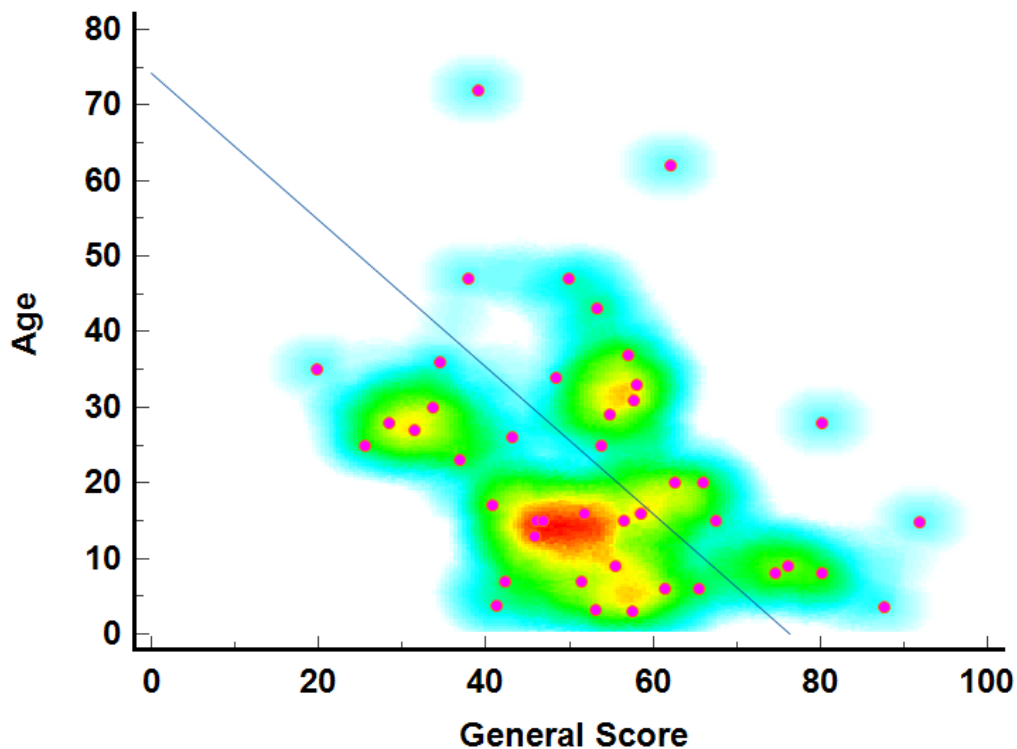


Figure 2. Heatmap of the Correlation Between Overall Score and Age

The average age at diagnosis was approximately 2.95 years, with most diagnoses occurring around the age of 2 years, reflecting a concentration of diagnoses in early childhood with little variability between cases. The Shapiro-Wilk test results for diagnosis also emphasized that the age at diagnosis does not follow a normal distribution. Still, one specific to this cohort highlights the need for age-specific approaches to treatments and interventions.

The study also evaluated various categorical variables related to SMA, such as the type of disease, the number of copies of the SMN2 gene, geographic location, patient



mobility, the need for enteral nutrition, and dependence on ventilation. It emerged that Type 2 SMA is the most common, affecting 60.4% of patients, followed by Type 3 and Type 1, the latter being the most severe.

Geographically (Figure 3), most patients reside in urban areas, reflecting better access to medical facilities. Regarding mobility, most patients cannot walk, which illustrates the severe impact of SMA on motor function. A relatively small percentage of patients require enteral nutrition, indicating a relative ability to manage feeding normally despite the severity of SMA. In terms of mechanical ventilation, over half of the participants require this type of support, underscoring the disease's impact on respiratory function.

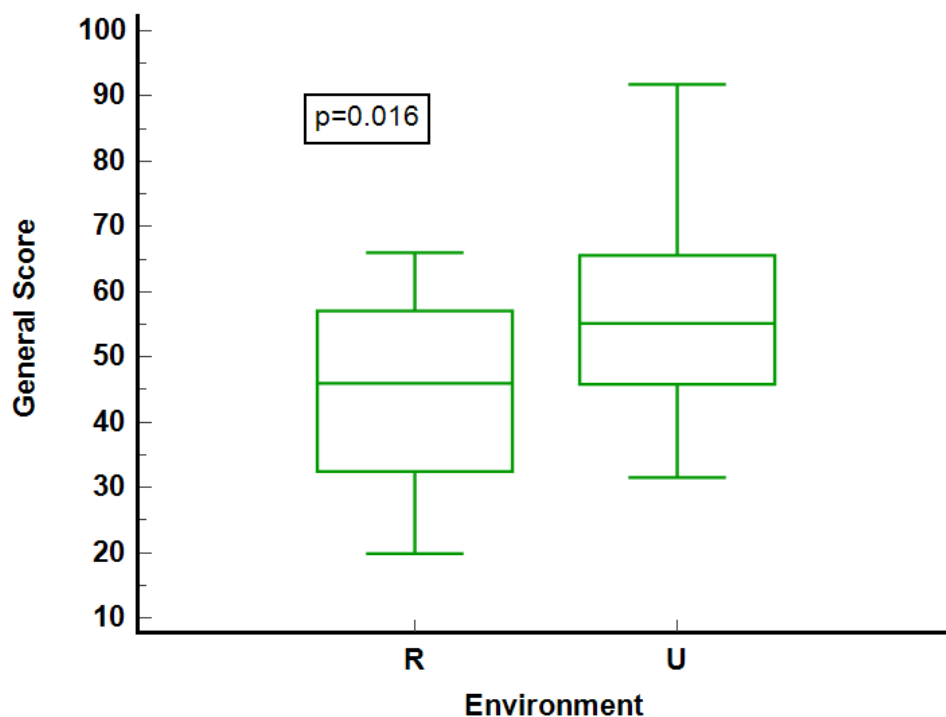


Figure 3. Overall score stratified by place of origin

These results highlight the complexity of AMS and underscore the need for a personalized approach in the management and treatment of this condition, providing valuable data for a deeper understanding of the disease and for improving intervention strategies. This information is essential for optimizing clinical outcomes for patients affected by this debilitating condition and emphasizes the importance of continuing research to include a broader spectrum of the population and to address the specific needs of different subgroups of AMS patients.

## **ELEMENTS OF ORIGINALITY AND PERSONAL CONTRIBUTIONS**

The first significant contribution of this work is the pioneering application and validation of complex genetic and clinical-therapeutic analysis methods for AMS patients in Romania, highlighting the specific genetic characteristics of this population. Additionally, the thesis brings innovations by developing and implementing new treatment protocols based on recent discoveries in gene and pharmacological therapy aimed at personalizing treatment for patients according to their specific genetic and clinicopathological profiles.

Another innovative aspect is introducing and adapting a functional assessment scale for AMS patients, adjusted to the medical and socio-cultural context of Romania, allowing for more precise and effective monitoring of disease progression and treatment response. These contributions reflect a deep commitment to improving the care of AMS patients and provide a solid foundation for future research and the development of public health policies in this field.

## **LIMITATIONS OF THIS DOCTORAL THESIS**

This doctoral thesis provides essential insights into the management of AMS but has several limitations. First, the small sample size, typical of studies on rare diseases, may affect the generalizability of the results, as it does not represent the entire population of spinal muscular atrophy patients in Romania. This could limit the applicability of the findings in other contexts or subpopulations. Second, the geographic focus on urban centers may overlook the particularities and needs of rural patients who may have different access to treatments and medical services. This introduces a potential bias in the interpretation of the data and the formulation of health policy recommendations. Additionally, the absence of a control group may lead to misinterpretation of the efficacy of therapeutic interventions, as it does not allow for comparison with a group that does not receive the experimental treatment. Finally, the study acknowledges the need for further research to refine therapeutic approaches, suggesting that the current understanding of the disease and treatment options is incomplete. These limitations underscore the importance of expanding research to include more extensive and more diverse samples and implementing study designs that allow for more robust evaluations of clinical interventions.

## GENERAL CONCLUSIONS

The studies included in this doctoral thesis, spanning five years of research, have provided crucial insights into the management of spinal muscular atrophy (SMA) and related aspects:

The study presented in the thesis is divided into two essential phases, focusing on the effects of nusinersen treatment on motor function and quality of life for patients with type 2 and type 3 SMA.

The first study, conducted on 37 adult patients over 54 months, focused on evaluating the impact of nusinersen on motor function, using validated scales such as RULM and HFSME.

The second study analyzed the effects of treatment on the quality of life of pediatric and adult patients, using the SF-36 questionnaire at the start of treatment and six months after. This research provided valuable insights into the treatment's benefits and its methodological limitations, including sample size and dropout rates.

Both studies highlight the significant improvements brought by nusinersen in motor function and quality of life, emphasizing the influence of demographic and genetic factors on treatment outcomes. These findings demonstrate consistency with the existing literature and the need for continued research to refine and expand the therapeutic applications of nusinersen, with a particular focus on tailoring treatments to patient's genetic and demographic particularities.

In summary, this doctoral thesis significantly contributes to the field by addressing critical aspects of SMA management. These findings provide a foundation for developing personalized therapy strategies and improving outcomes for individuals living with SMA.

## **FUTURE STUDIES**

In the future, I intend to advance research in this rare disease—spinal muscular atrophy. My research will explore personalized therapies and innovative treatment strategies, considering the genetic variations among patients. It is necessary to expand sample sizes to include diverse populations and age groups to evaluate the long-term effectiveness of treatments (including through neurofilament testing) and to better understand the impact of early interventions, such as a national neonatal SMA screening program. Additionally, the research would benefit from international collaborations to improve treatment protocols and integrate new genetic and molecular discoveries into clinical practice. This will significantly contribute to personalizing care and optimizing outcomes for SMA patients.