Immediate and Sustained Effects of Intensive Equine-Assisted Physiotherapy Based on Neuro-proprioceptive “Facilitation and Inhibition” on Psychomotor Development, Clinical Functions, Quality of Life, and Molecular Biological Indicators in Children with Spinal Muscular Atrophy: Protocol for a Randomized Controlled Trial

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Abstract

Background: Spinal muscular atrophy (SMA) is a rare neuromuscular disease and the most common genetic cause of infant death. Although pharmacological treatment improves survival rates and functional capacity, physiotherapy remains a key component of care. A newly developed innovative equine-assisted physiotherapy method based on neuro-proprioceptive “facilitation and inhibition” principles (NEUROEQUIP-SMA), could potentially improve the quality and extent of motor development in children with SMA.

Objective: The aim of the study is to compare NEUROEQUIP-SMA with standard physiotherapy and to evaluate its effect on functional outcomes and quality of life. In addition, the response of molecular biomarkers, such as long non-coding RNAs (lncRNAs), to treatment will be monitored.

Methods: In this randomized, assessor-blinded controlled trial (NCT05341453), 16 children with SMA types I–III (aged 2–9 years) will be divided into two groups. Both groups will undergo a 6-day therapeutic program of equal duration and intensity. The intervention group will undergo NEUROEQUIP-SMA therapy, the control group will undergo standard physiotherapy, and both groups will undergo therapeutic grooming. Primary outcomes include motor coordination measured using 3D motion analysis, the presence of muscle fatigue (using surface EMG), spirometry, standardized tests and scaling tests. Secondary outcomes include monitoring of children's psychomotor development (home video analysis) and quality of life (the International Classification of Functioning, Disability and Health-based Documentation Form for the category "Children with Cerebral Palsy Brief”). LncRNAs will be analyzed from blood samples. Statistical analysis will be performed in R software. The study was approved by the Ethics Committee of the Third Faculty of Medicine, Charles University (ref.: 24.2.2022 and 6.3.2023). Informed consent was obtained from the legal representatives of the participants.

Results: The study was initially designed for 8 participants. Data collection began in April 2022 and was completed in October 2024. Data analyses are planned for autumn 2025. Study results are expected to be available in 2026. The study is anticipated to be fully completed by the end of 2026.

Conclusions: This trial will be the first study to evaluate the effects of an equine-assisted physiotherapy intervention based on neuro-proprioceptive principles in children with SMA. If the intervention proves effective, it could expand physiotherapy options

for this new generation of children receiving genetic therapies. It is also expected that the results of this study may contribute to the optimization of rehabilitation strategies that integrate biomechanical, clinical, and molecular perspectives. Clinical Trial: ClinicalTrials.gov NCT05341453; https://clinicaltrials.gov/study/NCT05341453.

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A short title
Neuro-proprioceptive Equine-assisted Physiotherapy for Spinal Muscular Atrophy

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Trial registration: ClinicalTrials.gov NCT05341453**;** https://clinicaltrials.gov/study/NCT05341453**.**

International Registered Report Identifier (IRRID):

Keywords: Muscular atrophy, Spinal; Genetic Therapy; Equine-Assisted Therapy; Child; Pediatrics; Physical Therapy Modalities; RNA, Long Noncoding; Neuro-proprioceptive Principles

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Introduction

Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by the loss or mutation of the *SMN1* gene, with retention of its paralog *SMN2*. Despite the latest treatment options, it is the leading genetic cause of infant mortality under two years of age and affects approximately 1 in 10,000 live births in the Czech Republic [1].
The primary characteristic of SMA is the degeneration of alpha motor neurons in the anterior horn of the spinal cord [2]. This leads to a wide range of clinical manifestations, from severe neonatal weakness and respiratory failure to mild proximal weakness presenting in adulthood [3,4]. Although new pharmacological advancements are altering SMA phenotypes and subsequently the clinical manifestation of the disease [5,6], multidisciplinary supportive care remains essential to achieve optimal outcomes [2,7] , with physiotherapy (PT) being an integral part of it.
The perspective on PT has been rapidly evolving in recent years. In the past, it was prohibited, as it was assumed that it might accelerate the degeneration of diseased muscles [8,9] . This non-interventionist approach proved to be ineffective, as it deepened muscle weakness and fatigue, leading to mobility impairments [2,10]. Over time, interventions such as positioning, breathing exercises, range-of-motion activities, and targeted strengthening began to be used to prevent complications, e.g., contractures and deformities. Nowadays, the focus has shifted more towards enhancing functional mobility, utilizing techniques such as balance training, developmental activities, and gait rehabilitation [11–14].

Experimental research [15] showed a close link between neuron-specific protection and their activation levels by different exercise parameters. Exercise-induced neuroprotection was independent of SMN protein expression and was associated with specific metabolic and behavioral adaptations, including a reduction in muscle fatigability. This new insight into the motor units' adaptations significantly influenced and motivated us to develop a new active physiotherapy approach for children with SMA. We decided to utilize the principles of neuro-proprioceptive "facilitation, inhibition" in therapy, in which it is assumed that, through the combination of appropriate afferent stimuli, the interneuronal system is activated, allowing the transmission of missing signals and enabling the activation of movements that the patient would otherwise be unable to consciously perform. This approach is used in the Czech Republic for neurologically impaired individuals, such as post-stroke individuals [16] or people with multiple sclerosis [17]. However, it has not yet been studied in children with SMA.

We have developed a unique method of Equine-Assisted Physiotherapy Based on Neuro-proprioceptive "Facilitation and Inhibition" (NEUROEQUIP-SMA). Standard Equine-Assisted Physiotherapy has the potential to influence multiple domains, including posture, balance, and coordination, through the dynamic and repetitive movement provided by the horse.[18–20] It has been demonstrated that, in children with cerebral palsy, it shows benefits such as improved postural control and reduced spasticity [18,21,22]. In children with SMA [23], it leads to physical improvements (enhanced muscle strength and better balance) and psychological improvements (self-confidence, self-esteem, pride, independence, and a sense of achievement).

This study primarily aims to compare the effectiveness of two physiotherapy approaches: NEUROEQUIP-SMA and a standard physiotherapy protocol. We hypothesize that

NEUROEQUIP-SMA will have a greater impact on functional and postural outcomes in children with SMA due to:

1. The rider moving in harmony with the horse's motion rather than resisting it as in conventional stabilization techniques;
2. Simultaneous activation of multiple muscle chains, enabling coordinated engagement of weakened muscles;
3. Afferent input combination that initiates movement from the pelvis, lowers the center of gravity, supports the diaphragm in an expiratory position, relaxes the shoulder girdle, and improves overall stability;
4. Increased sensory input intensity and greater movement complexity, further promoting neuromuscular activation.

Primary Hypotheses

Immediately after the six-day intensive program, we expect improvements in both groups; however, we anticipate more pronounced effects in the intervention group, including:

1. No undesirable muscle fatigue (assessed using surface electromyography, sEMG).
2. Improved coordination of the torso and cervical spine, improved weight shift control and deeper breathing in the abdominal part of the torso.
3. Same or improved scores on the Hammersmith Functional Motor Scale for children with SMA and Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders.
4. Improved spirometry measurements.
5. Improved performance in the Deep Neck Flexor and Neck Extensor Endurance tests.

Secondary Hypothesis

We anticipate improvements in both groups; however, the intervention group is expected to demonstrate a greater enhancement in quality of life—assessed using the ICF-based Documentation Form for the Brief Category of Children with Cerebral Palsy—as well as a more substantial increase in both the quality and scope of psychomotor development, evaluated through home video recordings conducted 28 days after completion of the therapeutic program. Moreover, molecular biological markers are expected to change immediately after the completion of the therapeutic program.

As a complementary and innovative approach to document the benefits of equine-related activities, monitoring will be complemented by analysis of long noncoding RNAs (lncRNAs) expression in peripheral blood. These 200+ nucleotide long transcripts (e.g. SMN-AS1) are involved in the regulation of gene expression, e.g. through association with polycomb repressive complex 2 (PRC2) ([24]. Their deregulated levels are associated with many pathological conditions including neurodegenerative diseases. Changes in the expression of selected lncRNAs in body fluids may also reflect changes in the body in response to therapeutic intervention [25]. We propose a set of selected lncRNAs as a biomarker of improvement status in SMA patients after the inclusion of intensive therapy. Based on previous studies, we selected the following lncRNAs: MALAT1 and PARTICLE, which interact with PCR2 [26]. This makes them similar to SMN-AS1, which inhibits SMN2 expression by binding PCR2 [24]. MEG3, MALAT1, NEAT1 are lncRNAs involved in motor neuron development [27], which are affected in this disease. Deregulated levels of the

lncRNAs GAS5 and H19 have been detected in the blood of patients with neurodegenerative disease [25]. Analysis of selected lncRNAs in the blood of SMA patients before and after intensive therapy could provide novel diagnostic and prognostic biomarkers, therapeutic targets, and finally to understand the neurophysiological effects of the treatment.

Methods

Study design

This study is designed as a single-blind randomized controlled trial, registered under ID: NCT05341453 in the ClinicalTrials.gov database (https://clinicaltrials.gov/study/NCT05341453). Participants will be children with SMA types I, II, and III, aged two to nine years, who will be randomly assigned to two groups. Both groups will undergo a six-day therapeutic program of equal length (30 minutes of individual treatment per day) and with the same therapeutic goal. Subsequently, both groups will take part in therapeutic horse grooming (20 minutes per day). The groups will differ in the form of therapy: the intervention group will receive a newly developed Equine-Assisted Physiotherapy based on Neuro-proprioceptive “Facilitation and Inhibition”, while the control group will undergo standard individual ambulatory physiotherapy. Primary outcomes will be assessed before and at the end of the therapy program. These will include muscle fatigue assessment using surface electromyography, as well as functional scales and clinical tests measuring the strength of neck extensors and flexors, spirometry measurements and special tests recorded using 16 Qualisys Motion Capture System cameras. Secondary outcomes will be evaluated before and on the 28th day after the program, incorporating a quality-of-life questionnaire and home video recordings for movement quality analysis. To monitor molecular biological markers of rehabilitation, long non-coding RNAs (lncRNAs) will be analyzed (Figure 1).

	STUDY PERIOD						
	ENROLMENT	ALLOCATION	STUDY - ALLOCATION				
TIMEPOINT	0	0	t1	t2	t3	t4	t5
			DAY 1	DAY 2	DAY 7	DAY 8	DAY 36
ENROLMENT							
ELIGIBILITY SCREEN							
INFORMED CONSENT							
ALLOCATION							
INTERVENTION							
STANDARD PHYSIOTHERAPY							
NEUROEQUIP-SMA							
ASSESSMENTS							
BASIC CHARAKTERISTIC							
PRIMARY OUTCOME							
SECONDARY OUTCOME							
MOLECULAR BIOMARKERS							

Figure 1. Study design.

Study setting

Clinical examinations will be conducted at the College of Polytechnics Jihlava, which is equipped with advanced technology, including a 16-channel surface EMG system (DELSYS), spirometry, and Qualisys Motion Capture System with 16 cameras. The Mirákl Hippotherapy Center, a healthcare facility offering year-round Equine-Assisted Physiotherapy programs for children with special needs, has extensive experience and resources to support the study. LncRNA analysis will be performed at the Department of Medical Genetics, Third Faculty of Medicine, Charles University, which houses a state-of-the-art molecular biology laboratory specializing in genomic and biomarker research. The Institute of Computer Science at the Czech Academy of Sciences will contribute data management and statistical data analysis. Additionally, the Third Faculty of Medicine, Charles University, will provide institutional support for clinical research coordination, ensuring efficient execution and fostering interdisciplinary collaboration.

Ethics and dissemination

The multicentric ethics committee of the Third Medical Faculty of Charles University has approved the study under code (24.2.2022) and amendments (6.3.2023), based on submitted informed consent forms. The anticipated end of data collection is November 2025. The results of the study will be disseminated to the public and the scientific and medical community (published in journal articles and conferences). Anonymized data and outcome data may be published for research purposes (for meta-analysis, systematic reviews, etc.) on request. This study does not include a Data Monitoring Committee (DMC), as the intervention is short-lasting, low risk, and non-pharmacological in nature. Oversight is ensured by the principal investigator and the ethics committee. Any modifications to the study design will be submitted to the ethics committee for review, and the informed consent form will be revised accordingly.

Research and management team

The team includes coordinators, molecular biologists, specialists in 3D motion analysis and surface electromyography (sEMG), physiotherapists—including those with expertise in Equine-Assisted Physiotherapy—horse trainers, and statisticians. The study coordinator will oversee recruitment, enrolment, eligibility assessment, and study conduct while providing participants with study details. All personnel involved in examinations will have no role in treatment allocation. A blinded assessor will perform clinical measurements, evaluate video recordings of clinical and functional tests, and collect questionnaire data. Molecular biologists will process peripheral blood samples for lncRNA analysis. Specialists will oversee 3D motion analysis, sEMG, and data processing. The statistician will manage dataset preparation, do statistical hypothesis testing and interpret their results.

Sample size

Due to the limited number of patients with SMA, the planned number of participants is 16 (8 children per group). This number is sufficient to detect a larger group-by-time interaction effect (Cohen's f = 0.75) on the primary quantitative outcomes, with a power of 80% and a significance level of 5%, using the two-way mixed analysis of variance (ANOVA). However, given the small sample size, a non-parametric approach, aligned with ranks transformation (ART) ANOVA, will be primarily applied. To our knowledge, precise sample size-based effect estimates are not available for ART ANOVA. Regardless of the ANOVA method, the analysis will have the statistical power to detect a larger therapeutic intervention effect if one actually exists. The calculation was performed using the wp.rmanova() function from the WebPower package, version 0.9.4, in R software (https://www.r-project.org).

Recruitment

Participants will be continuously recruited from the database of the patient organization SMÁci, z. s., which registers 90% of children with SMA in the Czech Republic, while the remaining participants will be sourced from the database of the Mirákl Hippotherapy Center, o.p.s. All parents of children who meet the entry criteria will be contacted. The children will gradually participate in therapeutic cycles, each time in a group of eight—four assigned to the intervention group and four to the control group. Data collection and evaluation are scheduled to continue until November 2025.

Eligibility criteria

Participants will be eligible if they are between two and nine years old and have a diagnosed spinal muscular atrophy type I, II, or III with a stable health condition for at least six months and no other serious illnesses. Exclusion criteria will include hip subluxation, allergies to horses or the stable environment, and an overwhelming fear of horses.

Randomization, blinding

The parents of children participating in the study will be fully informed. Upon giving written informed consent, their children will be randomly assigned to one of the treatment groups. Block randomization with block sizes of 2 and 4 will be used to achieve balanced group sizes, with allocation concealment maintained through opaque sealed envelopes. The randomization sequence will be generated using the online software Sealed Envelope. [28] Outcome assessors and investigators will remain blinded to minimize potential bias in outcome assessments. All examinations will take place in the same examination room under identical conditions, regardless of the assigned intervention. However, blinding will not be feasible for therapists, children with SMA, or their parents due to the nature of the interventions. To reduce bias, children and parents will be strongly advised not to disclose the allocated therapy during both the baseline and final assessments. While this is the planned procedure, it is acknowledged that practical factors—such as participant availability, medical status, or logistical constraints—may necessitate adjustments to the randomization or blinding processes to ensure the study's feasibility and ethical conduct.

Retention and withdrawal, participation conditions

To support adherence, therapists will use their expertise and provide effective instruction to tailor each therapy session to the individual needs of the child, thereby enhancing adherence. Reasons for participant dropout will include discontinuing therapy for more than two missed physiotherapy sessions or significant non-cooperation from the child. Participants will have the option to withdraw from the study at any time.

Permitted and prohibited concomitant care

During the 6-day intervention period, participants will not undergo any additional rehabilitation therapies. In the following 28 days, only standard outpatient physiotherapy will be allowed, with a maximum frequency of once per week.

Intervention

Both groups will participate in a six-day therapy program with the same duration of therapy (50 minutes per day) and therapeutic goals, as determined by the kinesiological examination. The key difference lies in the form of therapy: the intervention group will receive the newly developed Equine-Assisted Physiotherapy based on Neuro-proprioceptive "Facilitation and Inhibition" (15 minutes, twice daily), while the control group will undergo standard individual ambulatory physiotherapy (30 minutes, once daily). Following the therapy sessions, both groups will engage in therapeutic horse grooming for 20 minutes (Table 1, see Multimedia Appendix 1).

Table 1. Treatment protocol for Intervention and Control Groups.

Group	Intervention group	Control group
Frequency of treatment	50 min per day	50 min per day
Content of physiotherapy	Equine-Assisted Physiotherapy based on	Standard individual outpatient physiotherapy

	Neuro-proprioceptive “Facilitation and Inhibition”	
Length of therapy	15 min twice a day	30 min per day
Length of therapeutic grooming	20 min per day	20 min per day
Environment	Riding hall or outdoor terrain	Treatment room
Involvement of specialists	Collaboration of physiotherapist with a team including a horse trainer and leader	Physiotherapist only

Equine-Assisted Physiotherapy based on Neuro-proprioceptive “Facilitation and Inhibition” (NEUROEQUIP-SMA)

NEUROEQUIP-SMA is an individualized therapeutic method designed to activate proper motor function through the child’s-controlled response to the movement of the horse’s back, thereby improving the quality of movement. The goal is not to stabilize against the horse's movement but to accompany it, facilitating natural reactions primarily in the pelvic area. The child's movement is secondary, naturally distributed, and rhythmically coordinated throughout the body.

The therapy involves careful selection of the horse, therapeutic equipment, appropriate terrain, horse-leading technique, stride length, movement speed, horse head position, child’s position, use of positioning aids, and the physiotherapist’s manual contacts. The horse is chosen based on specific movement patterns that facilitate the desired motor responses. The pace, stride length, and direction of the horse's movement are individually adapted to the child's needs, with the therapist actively facilitating movement and correcting the child's position using manual contacts or various types of positioning aids (see Multimedia Appendix 2). The child is placed in various positions (sitting, lying, assisted sitting) according to therapeutic goals and current capabilities.

The intervention is performed by a physiotherapist who is specially trained and certified in equine assisted physiotherapy. Professional competence is obtained by completing a course accredited by the Ministry of Health of the Czech Republic [29,30] and working under professional supervision. The physiotherapist is part of a multidisciplinary team alongside a horse trainer and a horse leader. The horse must be specially trained for therapeutic purposes.

Therapy sessions are conducted either in a therapeutic riding hall or in an outdoor arena with equivalent surface conditions and a calm environment. The intervention is individually tailored based on the clinical assessment of the child, their current motor abilities, and therapeutic goals. Adjustments to meet therapy objectives may include changing the horse, modifying stride length, movement speed, horse-leading method, use of aids, child’s position, or the intensity of the therapist’s facilitation. Therapeutic tolerance is monitored through control or final examinations and feedback from parents. The success of the

intervention is assessed based on the achievement of short-term therapy goals.

Standard individual ambulatory physiotherapy

Therapy follows the recommendations of the International Coordinating Committee for SMA Clinical Trials – Standard of Care and is guided by regular clinical assessments. It is tailored to meet the individual needs of each child, considering their motor abilities and disease progression. The primary goals are to maintain or improve muscle strength, prevent contractures and deformities, support postural control, and optimize respiratory function. Therapeutic interventions include regular positioning, passive and active range of motion exercises, facilitation of functional movement patterns, and targeted training designed to enhance both stability and mobility [12].

For the purposes of the study, key principles for addressing functional problems in both the intervention and control groups were established (Table 2).

Table 2. Key Principles for Addressing Functional Problems in the Intervention and Control Groups.

Functional problems in children with SMA	Standard individual physiotherapy	NEUROEQUIP-SMA
Contractures	Stretching	Chest area relaxation
Limited lung capacity	Breathing exercises	Differentiation in the transverse plane – supporting exhalation
Hip joint dislocations	Positioning	Differentiation in the sagittal plane – pubic bone area relaxation
Scoliosis	Exercises on unstable platforms	Differentiation in the frontal plane – weight transfer symmetry
Weakness of flexors and extensors (CP)	Active exercises	Assisted sitting position – correction of the chest area
Upper and lower limb strength	Active exercises	Differentiation in the sagittal plane – lowering the center of gravity to the pelvic area
Movement quality	Assisted movement	Activation of the pelvis as a movement initiator
Movement quantity	Exercise on unstable platforms	Using the horse’s back as an unstable platform

Development of walking	Motomed, Thera-Suit	Activation of the pelvis as a movement initiator

Therapeutic grooming

Therapeutic grooming is a psychosocial intervention that combines physical contact with a horse, sensory stimulation, movement therapy, and emotional regulation. This method includes activities such as brushing the horse, leading it by hand, stroking, and other forms of physical interaction. These activities allow children to engage naturally with the horse in a safe and supportive environment, promoting both physical and psychological development.

The therapy is guided by a trained therapist, who tailors the intervention to the individual needs of the child. This process involves selecting a suitable horse, adapting the environment, choosing appropriate aids, and applying specific touch techniques, all aimed at maximizing the therapeutic benefits. The goal extends beyond merely brushing the horse's coat to stimulating sensory perception, regulating emotions and muscle tone, and enhancing body awareness.

Sessions take place in a calm and welcoming environment, where external noise and stressors are minimized, facilitating relaxation and allowing the child to focus on the present moment. The effectiveness of the intervention is evaluated through observations of the child’s interaction with the horse, emotional state before and after therapy, changes in motor skills, and long-term improvements in self-confidence, communication, and stress management.

Overall, therapeutic grooming offers a unique blend of physical care and emotional well-being. Through their connection with the horse, participants experience deep relaxation, inner balance, and the development of vital life skills.

Data collection and management

To support data consistency, instruments are calibrated regularly. A statistician and coordinator perform data checks. To ensure data collection, an Excel form will be created, and basic statistical reports will be generated regularly to check the completeness and validity of the data. Personal data will be pseudonymized using unique subject codes and stored on password-protected computers. Only the research team will have access to the identifiable data, and all records will be kept confidential in accordance with GDPR regulations. Anonymized data may be used for analysis and shared only as permitted by the informed consent.

Outcome measures

Demographic and anamnestic data will be collected, including age, gender, information about the disease and social anamnesis, the start and type of medication or rehabilitation, and use of compensatory aids prior before the program.

Primary outcome measures

3D Motion-Based Evaluation of Postural Control, Breathing, and Coordination

All three tests share a common setup, in which the child sits straddling a cylinder with sensors attached. The cylinder is positioned within a calibrated 3D space and firmly secured to prevent movement. The child sits with feet on the ground and hips flexed to at least 90 degrees. Eighteen markers are attached bilaterally at anatomically significant points, including the inferior angle of the scapula, sacroiliac joint, distal end of the clavicle, temporomandibular joint, dorsum of the hand, 4th rib (one-quarter proximal to the sternum), 10th rib (at the midpoint), top of the scapula, and hip. Additional markers are placed unilaterally at C7 – the thoracolumbar junction (Th/L), L4– the distal end of the sternum, and the umbilicus (see Multimedia Appendix 3). Marker motion is captured using sixteen Qualisys Motion Capture System cameras, allowing calculation of their spatial coordinates, from which position, velocity, and acceleration data are derived. Each measurement session lasts 30 seconds.

Test of Trunk and Cervical Spine Coordination Ability

The child performs repeated back-and-forth upper limb movements while sitting on the cylinder, typically 3–6 repetitions. Key variables include the extent of thrust from the body's center, the point at which cervical spine extension begins as compensation, and the coordination between markers located on the dorsum of the hand, C7 vertebra, and temporomandibular joint. A higher functional level is reflected by the child's ability to synchronize cervical spine movements with trunk and upper limb actions over a longer period without compensatory cervical spine extensions (see Multimedia Appendix 4a).

Abdominal Breathing Ability Test

Over a 30-second period, the child is instructed to breathe deeply, aiming for 8–12 respiratory cycles. Analysis focuses on the displacement of markers on the distal clavicles, distal sternum, L4, and the umbilicus during inspiration and expiration. The objective is to detect shifts toward an abdominal breathing pattern, with improved function indicated by increased distance between the Th/L and L4 markers relative to those around the umbilicus.

Ability to Work with the Centre of Gravity

During the measurement, the child is asked to extend one upper limb sideways, and, depending on cooperation, may be encouraged to reach for a toy. Movements are performed 3 to 6 times within the 30-second recording period. This test evaluates stability and control over the center of gravity, with progress reflected in greater deviations of the markers from their initial positions after therapy (see Multimedia Appendix 4b).

Muscle fatigue

Muscle fatigue will be monitored using sEMG. A 16-channel surface EMG system from DELSYS will be used to measure the activity of the m. obliquus externus abdominis for 30 seconds. The child will lie on their back, and the therapist will present a toy near the opposite knee to encourage the child to reach for it. The movement will be performed first in

an isometric contraction, followed by a repetitive movement. We expect the child to be able to repeat the movement 5–10 times during the 30-second measurement period. The EMG data will be processed using standard methods and normalized to the maximum EMG observed during isometric contraction to compare data between different muscles (right and left side of the child). A lower median frequency and decreasing EMG amplitude during repeated movement indicate improved function, as the muscle performs the task more efficiently and with less effort. Additionally, a higher number of repetitions without significant changes in EMG characteristics reflects better function, suggesting functionally stronger or more coordinated muscle activation.

The expanded Hammersmith Functional Motor Scale

The assessment tool, designed to evaluate motor function in children with neuromuscular disorders, consists of 33 items that assess a range of functional movements, including both gross and fine motor skills. It is used to monitor changes in motor function over time and to evaluate the effectiveness of interventions [31]. A higher score indicates better quantity of motor skills.

Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders

A clinical assessment tool used to evaluate motor function in infants with neuromuscular disorders measures their ability to perform specific movements and assesses the severity of motor impairments. Measurements are recorded during the test and are later evaluated by two independent physiotherapists [32]. A higher score indicates a larger scope of motor skills.

Deep Neck Flexor Endurance

The child lies on his back, legs extended and slightly supported under the knees. The arms are held loosely along the body. The child's head is in a neutral position. Task: The child should gently pull the chin towards the chest, thus activating the deep neck flexors, without moving or securing with the hands. At the same time, he should hold this position for as long as possible, without any head movement or deviation from the neutral position. The test is stopped when the child cannot keep the head in the desired position or begins to compensate by moving the trunk or other parts of the body. The time in seconds for the child to maintain the correct position is measured [33]. The time is supplemented by a qualitative assessment based on a video recorded by two independent physiotherapists. Higher endurance time and better quality indicate better function of the deep neck flexors.

Neck extensor endurance test

The child lies on the couch on stomach, the head and cervical spine are outside the couch. The arms are loose along the body with the palms up. The therapist holds the head in a neutral position. Task: To tilt the head back and hold this position for as long as possible without compensating with other parts of the body. The time the child holds the desired position (in seconds) is measured [34]. The endurance will be complemented by a qualitative assessment based on recorded video, evaluated by two independent physiotherapists. A longer holding time with higher quality indicates better function of the neck extensors.

Spirometry measurement

Spirometry will be performed according to the international standards of the European

Respiratory Society [35]. The patient will be fitted with a nose clip and then breathed into the spirometer through the mouthpiece while sitting on the cylinder. Each child receives the same instructions: Take a deep breath and then exhale as quickly and forcefully as possible. The test will be repeated 3 times in a row. It will be performed using the Vitalograph Asma-1 device. Forced expiratory volume in one second (FEV1) and peak expiratory flow (PEF) will be measured [36]. Higer values indicate better function.

Epigenetic examination

All blood will be taken on an empty stomach for RNA analysis. The A RiboPure ™ –Blood Kit (cat # AM1928, ThermoFisher Scientific) will be used to isolate high quality RNA directly from all the blood. This kit contains RNAlater® Solution (cat. # AM7020, ThermoFisher Scientific), which protects RNA and is designed to allow later processing of samples (not immediately after sampling), which is more processable. RNAlater® Solution also blocks a given cell gene expression profile. Samples treated in this way can be safely stored at room temperature for longer periods of time (up to three days or more). Blood samples stored in RNAlater® Solution achieve RNA quality comparable to the quality of samples processed immediately according to commercial websites. The expected average total RNA yields will be about 1 µg / 0.5 ml of all blood. Total RNA will be transcribed into cDNA by reverse transcriptase. To avoid error, the expression of human lncRNA and the internal endogenous gene (e.g., GAPDH) will be measured using RNA obtained from blinded samples (i.e., concealment of sample origin allocation). Blood collection will always take place in the evening before and after the complete end of the rehabilitation course, directly at the place of accommodation of families with children with SMA [37].

Secondary Outcome Measure Information:

Quality of life questionnaire

Parents of children with SMA will complete a special questionnaire at the beginning and 28 days after the end of the rehabilitation. The ICF score set, specifically the ICF-based Documentation Form for the category "Children with Cerebral Palsy Brief" (for children under 6 years old), will be used to evaluate the quality of life [38]. Although the ICF-based Documentation Form was originally developed for children with cerebral palsy, it was selected in this trial due to its better applicability in clinical practice and its ability to capture functional and quality-of-life domains relevant also to children with SMA.

Home video recording

At the beginning of the rehabilitation program and on the 28th day after its completion, parents will send home video recordings of their child's movement behavior. The video recordings will be evaluated by 2 independent physiotherapists who will assess quality and extent of the children's movement. Movement of greater quality or scope indicates better function.

Statistical analysis and software

Nonparametric tests will be primarily used to analyze quantitative outcomes, as normality cannot be assumed, and the sample size is too small to reliably test for normality and other assumptions of parametric tests. Differences in quantitative outcomes between groups will therefore be analyzed using the unpaired two-samples Wilcoxon test (also known as Wilcoxon rank sum test or Mann-Whitney test), and within groups using the paired samples Wilcoxon test (also known as Wilcoxon signed-rank test). The ART ANOVA will be used to summarize the effect of group (type of treatment), time (pre- and posttreatment

measurements) and their interaction on the quantitative outcomes. Fisher's and McNemar's tests will be performed to analyze the qualitative outcomes between and within groups, respectively. When doing multiple comparisons, the achieved level of significance (p-value) will be corrected using the Bonferroni method, or another appropriate correction method, to decrease the probability of a type I error. The p-value less than 0.05 will be considered statistically significant. Along with the p-value, the effect size will be reported. The R software will be used for statistical data analysis. Missing data will not be imputed. No interim analyses are planned. The trial will be conducted in full as described, unless terminated early for ethical or safety reasons upon recommendation of the principal investigator or the ethics committee.

Harms

The physiotherapists are qualified professionals who actively approach all subjects with respect to their individual impairment. The horses performing the therapy in the study have specialized examinations, and the trainers are professionals with many years of experience to sufficiently ensure safety during NEUROEQUIP-SMA and therapeutic care. The program is individualized to avoid burdening or harming the patient. All adverse events must be reported to the principal investigator and the appropriate specialist. All adverse events (AEs) will be monitored and systematically recorded by the treating physiotherapists during the study using a predefined reporting form. AEs will be classified according to severity (mild, moderate, severe) and potential relationship to the intervention (unrelated, possibly related, definitely related) and will be reviewed by the principal investigator and a designated clinical specialist. Serious adverse events will be reported immediately to the ethics committee. In the event of any injury related to the intervention, appropriate clinical care will be provided, but no financial compensation will be provided.

Results

In November 2021, all potentially eligible study participants - children aged 2-9 years - were contacted. Data collection ran from April 2022 to October 2024.
The statistical analysis is not yet underway and is planned to start in September 2025. The study is funded by the Grant Agency of Charles University (4424; January 2024), Programme Cooperatio (Neurosciences) and SVV 260533/SVV/2024.

Discussion

Principal results

This protocol presents the design of a clinical trial comparing an innovative NEUROEQUIP-SMA approach with standard physiotherapy in children with spinal muscular atrophy (SMA). This is the first study of its kind to evaluate the effect of EAT based on the principles of neuro-proprioceptive facilitation and inhibition. The uniqueness of the study also lies in the specific target group: children who have undergone gene therapy.
In the Czech Republic, the Nusinersen substitution therapy has been included in the system of reimbursed care as regular reimbursement since 2018 [39], while the Zolgensma gene therapy was approved by the Ministry of Health of the Czech Republic in April 2020 before registration as pre-authorized use [40]. Physiotherapists are thus in current practice dealing

with diverse groups of children: those treated with substitution therapy for a long time, those treated with a combination of both approaches and those who have received gene therapy very early. We have a new generation of children who can be expected to slow or stop the progression of the disease. [41,42]
Based on these changes, there is a need to revise existing rehabilitation strategies in clinical practice. Some physiotherapists are recommending an increase in the frequency and intensity of therapy [43] and new standards of care are needed to better reflect patient expectations and variable motor skills [44]. Although pharmacological treatment significantly improves survival, motor function often remains limited and is accompanied by pathological compensatory mechanisms [12,45,46]. Targeted correction of these functions with appropriate rehabilitation approaches is therefore essential. For this reason, an intensive 6-day course and the therapeutic neuro-proprioceptive principle were chosen.

Comparison with Prior Work

There is currently only one study examining the effect of EAT in children with SMA. Lemke et al. [23] focused on subjective assessment of the effects of EAT using a questionnaire survey. In contrast, our study investigates the direct response to a therapeutic intervention, which we are able to observe at the clinical, biomechanical and molecular levels, including comparison with a standardized SMA-SoC procedure.
Research on conventional rehabilitation approaches for children with SMA has so far focused mainly on maintaining existing function, preventing contractures and promoting self-sufficiency.[6,12,47] Most of these were designed before the onset of gene therapy, when disease progression was considered inevitable, and often lacked targeted monitoring of compensatory mechanisms. In our study, we augmented standardized tests with qualitative assessments to capture potential re-education of pathological patterns. In the era of genetic treatments, we consider the monitoring of movement quality to be essential, and the existing literature has not yet addressed this milestone.
Cammarano et al. [48] summarize that rehabilitation care for SMA, whether before or after genetic treatment, lacks standardization, high-quality randomized trials, and comprehensive multidomain evaluations. Our study addresses this requirement: it includes children treated with both replacement and genetic therapy.

Limitation of this study

As the participants are children, the results are partly influenced by their level of cooperation and willingness to participate. Although we carefully selected the assessment tools to minimize this influence, some degree of bias cannot be excluded, especially in the deep neck flexor endurance and neck extensor endurance tests. Limitations can also be seen in the selection of the quality-of-life test. Although the ICF tool is practical and most commonly used, it was originally validated for cerebral palsy, which may reduce its specificity for SMA.
The functional focus, specificity, precision, and technique inherent in the NEUROEQUIP-SMA methodology are key to the overall therapeutic effect and may contribute to minor differences in results.
Since SMA is a rare genetic disease, participants were recruited from across the country. The number of participants is limited by the rarity of the disease, which may limit the statistical power and generalizability of the results. Bias may also occur due to the established randomization process, where therapists and families could not be blinded.
Due to travel restrictions, the long-term effects of the NEUROEQUIP-SMA intervention could only be partially assessed. Furthermore, given the severity of the disease, it is likely

that children will engage in further physiotherapy interventions approximately four weeks after completing our treatment program.
Taken together, these limitations suggest that the results should be interpreted with caution and confirmed in larger, multicenter studies with extended follow-up.

Conclusion

The proposed evaluation methods provide a comprehensive assessment of the NEUROEQUIP-SMA approach and can serve as a foundation for future comparisons of different methodologies in equine-assisted therapy.
The findings of this study have the potential to inform and shape rehabilitation strategies for children with SMA receiving genetic or substitution pharmacotherapy. The results will be shared with the professional and lay public and translated into clinical practice.

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Patient and Public Involvement statement
No patients or public were involved in the design, development, or management of the trial.

Data Availability
The data collected during this study have not yet been analyzed. After the completion of data analysis, anonymized datasets will be made available upon reasonable request. Researchers interested in accessing the data may contact the corresponding author, and the data will be shared for scientific purposes.

Conflict of interests
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Disclaimer
The views expressed are those of the authors and do not necessarily reflect those of their affiliated institutions. The study is not intended to replace clinical judgment or individualized treatment recommendations.

Author’s note
The authors declare that all statements made in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations or the publisher, editors, and reviewers.
The SPIRIT 2025 protocol checklist is attached.

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Contributors and sources

Conception and design of the study – KŘ and KM; methodology of laboratory assessment – MV; methodology of therapy content – KŘ and KM; project administration – KŘ, KM, MP,

and MV; conceptualization and methods of lncRNA – MP and MČ; data set preparation, data management, and statistical analysis – JR; guarantor of the article – KŘ. All authors have read and approved the final version of the manuscript.

Abbreviations

lncRNAs	long non-coding RNAs
NEUROEQUIP-SMA	equine-assisted physiotherapy method based on neuro-proprioceptive “facilitation and inhibition” principles
PT	Physiotherapy
sEMG	Surface electromyography
SMA	Spinal muscular atrophy
ANOVA	analysis of variance
C7	seventh cervical vertebra
Th/L	thoracolumbar junction
L4	fourth lumbar vertebra
ICF	International Classification of Functioning, Disability and Health
FEV1	Forced Expiratory Volume in 1 second
PEF	Peak Expiratory Flow
AEs	Adverse events

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Supplementary Files

Untitled.
URL: http://asset.jmir.pub/assets/df59a6112567cc24ab14290b87f4c95f.docx

Multimedia Appendixes

Photographs illustrating different types of therapies: (a) equine-assisted physiotherapy based on neuro-proprioceptive facilitation and inhibition, (b) therapeutic grooming, (c) standard individual outpatient physiotherapy.
URL: http://asset.jmir.pub/assets/db243e30a6628824850bbee9af086189.docx

Example of a Key Therapeutic Element: Pelvic Positioning Correction: (a) Corrective position with correction by the therapist, the child is seated on the ischial tuberosity with external rotation of the hip joints, (b) The child presents with a pathological posture characterized by an anterior pelvic tilt and internal rotation of the hip joints.
URL: http://asset.jmir.pub/assets/c1b30ee2887930a9bc77581420ec6490.docx

Locations of the Eighteen Markers Used for Motion Analysis.
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Back-and-forth upper limb movements: (a) and sideways reaching, (b) while sitting on the cylinder.
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