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Short-Term Neural Recovery after Treatment for Spinal Cord Injury Using AXONIC-REVIVE™

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Abstract. The study is aimed to determine whether the AXONIC-REVIVE™ multimodal strategy resulted in measurable neural recovery following spinal cord injury, as assessed by a combination of functional parameters, neuroimaging data and laboratory markers. The study was conducted at the Faculty of Medicine of the University of Medicine and Social Sciences (Kharkiv) from 3 March to 31 December 2025, where the effectiveness of the applied strategy following spinal cord injury was assessed based on changes in the motor and sensory deficits, muscle tone, functional independence, as well as changes in the electrophysiological indicators of neuromuscular transmission and reinnervation, diffusion tensor magnetic resonance imaging (DTI-MPT) parameters of the conduction pathways, and concentrations of cerebrospinal fluid biomarkers of neurotrophic support, plasticity and glial response. The results showed that, by week 24, recovery was observed in both groups; however, it was more pronounced in the AXONIC-REVIVE™ group: the motor score increased by 18.6 points versus 11.2, the sensory score by 19.7 versus 11.0, spasticity decreased by 0.8 points compared to 0.3, functional independence increased by 20.7 points compared to 12.1, and an improved bladder control by week 24 was recorded in 57.1% of the patients in the main group compared to 32.1% in the control group. More favourable trends were also observed in electromyography (EMG) (F-wave latency decreased by 5.5 ms compared with 2.4 ms in the control group), diffusion tensor magnetic resonance imaging (fractional anisotropy increased by 0.09 versus 0.04) and cerebrospinal fluid biomarkers of neurotrophic support and plasticity compared with standard rehabilitation overall. Thus, up to week 24, the AXONIC-REVIVE™ multimodal strategy was associated with greater clinical and functional improvement compared with standard rehabilitation. The results obtained can be used by neurosurgeons, physiatrists and multidisciplinary teams in rehabilitation centres to justify comprehensive monitoring and the selection of combined approaches to neurorehabilitation following spinal cord injury in clinical practice and pilot protocols.

Keywords: rehabilitation, cerebrospinal fluid, electromyography, fractional anisotropy, collagen-laminin hydrogel, muscle tone, reinnervation.

Trumpalaikis nervų sistemos atsigavimas po nugaros smegenų traumos gydymo, taikant „AXONIC-REVIVE™“

Santrauka. Tyrimo tikslas buvo nustatyti, ar daugialypė strategija „AXONIC-REVIVE™“ lėmė išmatuojamą pacientų nervų sistemos atsigavimą po nugaros smegenų traumos, vertinant pagal funkcinių parametrų, neurovaizdinimo duomenų ir laboratorinių žymenų derinį. Tyrimas buvo atliktas Medicinos ir socialinių mokslų universiteto (Charkovas) Medicinos fakultete nuo 2025 m. kovo 3 d. iki gruodžio 31 d., kur taikytos

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po nugaros smegenų traumos strategijos veiksmingumas buvo vertinamas remiantis motorinių ir sensorinių sutrikimų, raumenų tonuso, funkcinio savarankiškumo pokyčiais, taip pat neuromiologinės transliacijos ir reinervacijos elektrofiziologinių rodiklių pokyčiais, difuzijos tenzoriaus magnetinio rezonanso tomografijos (DTI-MRT) parametrų laidumo takuose bei smegenų skysčio biožymenų, susijusių su neurotrofiniu palaikymu, plastiškumu ir glialinių ląstelių reakcija, koncentracijomis. Rezultatai parodė, kad iki 24-osios savaitės abiejose pacientų grupėse buvo nustatytas nervų sistemos atsigavimas; tačiau jis buvo ryškesnis „AXONIC-REVIVE™“ grupės pacientų: jų motorinių funkcijų balas padidėjo 18,6 taško, palyginti su 11,2, jutimo balas – 19,7, palyginti su 11,0, spastika sumažėjo 0,8 balo, palyginti su 0,3, funkcinis savarankiškumas padidėjo 20,7 balo, palyginti su 12,1, o 24-ąją savaitę šlapimo pūslės kontrolės pagerėjimas buvo užfiksuotas 57,1 % pagrindinės grupės pacientų, palyginti su 32,1 % kontrolinėje grupėje. Palankesnės tendencijos taip pat buvo pagal elektromiografijos (EMG) duomenis (F bangos latentinis laikas sumažėjo 5,5 ms, palyginti su 2,4 ms kontrolinėje grupėje), iš difuzijos tenzoriaus magnetinio rezonanso tomografijos duomenų (frakcinė anizotropija padidėjo 0,09, palyginti su 0,04) ir smegenų skysčio biožymenų, rodančių neurotrofinį palaikymą ir plastiškumą, palyginti su bendra standartine reabilitacija. Taigi iki 24-osios savaitės „AXONIC-REVIVE™“ multimodalinė strategija buvo susijusi su didesniu klinikiniu ir funkciniu pagerėjimu, palyginti su standartine reabilitacija. Gauti rezultatai gali būti naudojami neurochirurgų, fiziatrų ir daugiadisciplinių komandų reabilitacijos centruose, siekiant pagrįsti visapusišką stebėjimą ir kombinuotų neuroreabilitacijos metodų pasirinkimą po nugaros smegenų traumos klinikinėje praktikoje ir bandomuosiuose protokoluose.

Raktažodžiai: reabilitacija, smegenų ir nugaros smegenų skystis, elektromiografija, frakcinė anizotropija, kolageno ir laminino hidrogelis, raumenų tonusas, reinervacija.

Introduction

Spinal Cord Injury (SCI) causes persistent motor, sensory and autonomic impairments that limit ability to self-care, work and participate in social life, and result in a long-term need for medical care and rehabilitation. According to estimates by the World Health Organisation [1], over 15 million people worldwide live with the consequences of such an injury; therefore, even a moderate improvement in recovery can reduce the number of years lived with disability and the costs of care. Research into the mechanisms of neural regeneration is needed to justify biologically targeted interventions and select efficacy indicators. If these approaches are not developed, the incidence of secondary complications, chronic pain, infections, trophic disorders and depressive disorders will increase. This can cause permanent disability, loss of labour capacity, long-term dependence on external care and an increased risk of premature death.

Molecular events following SCI involve a reorganisation of neurotrophic support and changes in the peripheral motor system, which influence the quality of functional recovery [2–4]. In a study by A. Głowacka et al. [5], the consequences of an increased *Brain-Derived Neurotrophic Factor* (BDNF) expression in the spinal cord following injury were assessed at the neuromuscular junction. The study demonstrated that partial preservation of the synaptic structure is accompanied by changes in transcripts associated with cholinergic transmission, indicating a selective response depending on the muscle and motor neuron pool. The impact of reinnervation processes on recovery outcomes following nerve injury was analysed by K. Suszyński et al. [6], with an emphasis on how the restoration of functional connections determines the final motor outcome. The study concluded that delayed or incomplete reinnervation exacerbates persistent motor impairments even when anatomical integrity is technically restored; monitoring these processes is crucial for interpreting the effects of regenerative interventions. The role of antioxidant approaches in SCI was analysed by A. Nowacka et al. [7]. The study considered the reduction of oxidative stress as a means of influencing the cascade of secondary damage, in particular, inflammatory reactions and tissue degradation, which exacerbate neurological consequences.

Regenerative strategies for SCI require the creation of a restorative microenvironment in which biomaterials interact with biological signals to support cells and guide growth [8–10]. In a Polish study conducted by B. Niemczyk-Soczynska et al. [11], the properties of hydrogels, electrospun matrices and composite materials were compared. The researchers described how the mechanical parameters, porosity and bioactive motifs of the material determine cell adhesion, migration and neurite orientation, and influence the possibility of controlled delivery of molecules. Approaches to cell therapy for SCI, cell sources and criteria for assessing efficacy were summarised by B. Basiaga et al. [12]. The study concluded that the clinical response depended on the timing of administration, the method of delivery and the interaction of the graft with the inflammatory microenvironment, which directly influenced the safety and severity of the therapeutic effect. A. Głowacka [13] analysed the dynamics of molecular and morphological changes during stimulation of BDNF overproduction in the rat spinal cord. The period of greatest functional dynamics and the associated changes in marker expression and tissue organisation were identified, which made it possible to link biological shifts to windows of opportunity for recovery.

Assessing neural recovery requires tools that quantitatively reflect structural changes in pathways and the functional state of motor systems to accurately track the effects of interventions over time [14–17]. The relationship between *Diffusion Tensor Imaging* (DTI) parameters in cerebral sensorimotor regions and neurophysiological deficits was described by A. Zimny et al. [18]. The study determined that diffusion parameters correlate with the severity of impairments and may serve as markers of changes in neural networks associated with motor control. The dynamics of functional recovery of motor units in patients with incomplete SCI, according to J. Huber et al. [19], were assessed in the context of peripheral nerve electrotherapy. The study determined that neurophysiological indicators of the muscle response change in tandem with the functional status, making the method suitable for objective monitoring of rehabilitation interventions. The prognostic value of serum Nogo protein isoform (Nogo A) for recovery after injury was assessed by S. Waliullah et al. [20], who compared marker levels with clinical neurological dynamics. The authors demonstrated that higher concentrations of Nogo A are associated with poorer recovery outcomes; therefore, the marker was considered a tool for predicting and stratifying patients according to their expected response to treatment.

A lack of consensus exists in the scientific literature regarding the evaluation of comprehensive protocols that combine pharmacological modulation of inhibitory pathways, local biomaterial delivery and trophic support, whilst simultaneously monitoring objective markers of recovery. The study aimed to evaluate the efficacy of the AXONIC-REVIVE™ multimodal strategy for neuroregeneration following SCI based on functional, neuroimaging and laboratory criteria. The objectives of the study were as follows: to assess changes in the motor function and neuromuscular conduction based on the EMG data; to determine the dynamics of DTI-MRI parameters characterising the integrity of conduction pathways; to measure and correlate the concentrations of BDNF, NT-3, GAP-43, MAP-2 and glial fibrillary acidic protein (GFAP) in cerebrospinal fluid with clinical dynamics.

Materials and methods

Study design and setting

The study employed a prospective, randomised, controlled design with parallel groups and repeated measurements at baseline and during the follow-up. The study was based at the Faculty of Medicine of the University of Medicine and Social Sciences (Kharkiv, Ukraine), and the practical part was conducted at its clinical and rehabilitation sites, where patient examinations and follow-up were conducted. The study period ran from 3 March 2025 to 31 December 2025, as this timeframe was

sufficient for the sequential recruitment of patients, the initiation of intervention in the early recovery period following injury, and the completion of scheduled follow-up with control examinations over a period of at least six months.

Characteristics of the participants, sample selection and grouping

The study included 56 patients with SCI who were undergoing treatment and follow-up at the university clinic. The sample was formed by sequentially recruiting all individuals who met the inclusion criteria during a specified period, after which, they were divided into two groups at a 1:1 ratio by using simple randomisation with a random number generator. Recruitment was announced during consultations in the neurosurgery department and rehabilitation centre, as well as through information notices in the relevant departments of the institution. The main group (n=28) received AXONIC-REVIVE™ alongside standard rehabilitation; the control group (n=28) received standard rehabilitation without the experimental intervention (which included therapeutic exercise, kinesiotherapy, prevention of contractures and secondary complications, training in self-care skills, and symptomatic pharmacological support according to clinical indications). The participant age range was from 18 to 65 years, with a mean age of 39.6±12.4 years. The sample comprised 36 men and 20 women. The predominance of men in the sample corresponded to the general epidemiology of spinal cord injury, as men are more frequently affected by such injuries and have higher rates of prevalence and years of life lost due to disability [21]. During the follow-up period, 4 participants (2 from the main group and 2 from the control group) were lost to follow-up, mainly due to refusal of further invasive procedures. Data from these participants were completely excluded from the analysis, and the results were calculated only for those patients who completed the follow-up protocol.

The inclusion criteria were traumatic spinal cord injury at the cervical or thoracic level; incomplete injury with preserved function below the level of injury; a post-injury period of 14 to 45 days; stable general medical condition; written informed consent; and the ability to undergo MRI and lumbar puncture. Exclusion criteria were complete spinal cord injury, severe traumatic brain injury, active infection, coagulation disorders or clinically significant contraindications to invasive procedures, immunodeficiency states, pregnancy, contraindications to MRI, known hypersensitivity to components of the protocol, as well as prior use of gene-based or other experimental biological products within the last six months

Protocol for intervention in the main group

The AXONIC-REVIVE™ protocol comprised several biologically targeted components with different mechanisms of action, which were administered in a coordinated manner within the first month following the start of the intervention. The protocol was implemented by a multidisciplinary team, with administration and local application conducted by a neurosurgeon in collaboration with an anaesthetist, whilst further management and monitoring of recovery were coordinated by specialists from the rehabilitation unit. Procedures were conducted in a specialised clinical setting under aseptic conditions and with continuous monitoring of the patient's condition, and all interventions were recorded in the medical records as part of the research protocol.

The anti-inhibitory block was administered by using a humanised monoclonal antibody against Nogo A at a dose of 5 mg/kg body weight in the form of a solution in 0.01 M phosphate-buffered saline (PBS), pH 7.4, which was administered locally within the area of influence by using sterile disposable injection systems with a Luer-lock connector. For standard local injections, 1 ml BD Luer Lok (USA) disposable syringes were used. For the precise delivery of small volumes, calibrated Hamilton 700 series (USA) microsyringes with nominal volumes of 10 µl, 25 µl or 50 µl were used. Modulation of the RhoA, a small GTPase from the family of small Rho GTPases (cytoskeletal regulators) (Rho)

family (RhoA)-Rho-associated coiled-coil containing protein kinase (ROCK) cascade was achieved by using a nanomolecular ROCK inhibitor at a dose of 0.5 mg/kg in the form of a suspension in a collagen-laminin hydrogel, which served as a biomimetic carrier and local scaffold, with the hydrogel being applied locally as a matrix within the defect site to retain the active agents within the damaged area. Remodelling of the extracellular matrix in the glial scar area was conducted by using chondroitinase ABC (ABC) at a concentration of 0.1 IU/ml, at a rate of 1 to 2 ml of hydrogel per cm of the lesion length, which was incorporated into the hydrogel matrix according to the length of the lesion and the plan for local enzyme exposure. Neurotrophic support was provided by the adeno-associated virus serotype 9 (AAV9) vector encoding BDNF and NT-3 at a concentration of 1×10^{11} viral particles per ml; the injection volume was 10 to 20 µl per centimetre of lesion, and the injections were administered locally with the control of volume and distribution within the affected segment according to the extent of the defect, by using microlitre-class microsyringes for stable dosing of the specified volumes. Where indicated, the protocol was supplemented with cellular modulation in the form of mesenchymal stem cells (MSC) or differentiation cluster 133 (CD133)-positive cells at a concentration of 1×10^{6} cells per ml of hydrogel, which were applied as a cell suspension in the carrier matrix followed by local administration to the target area.

The laminin-collagen hydrogel was used as a delivery matrix, ensuring the spatial retention of bioactive agents, maintaining the local microenvironment and facilitating the gradual release of components within the target area; it also served as the primary carrier for topical application and, where necessary, for the injection of individual fractions. The timing scheme involved the sequential application of components: on days 0–3, priority was given to ROCK inhibition; on days 3–7, the anti-Nogo A component was administered; on days 7–14, chondroitinase ABC was applied to target CSPG; and on days 14–30, the neurotrophic phase was conducted by using BDNF and NT-3, with the possible addition of a cellular component, after which, the patients continued standard rehabilitation and scheduled instrumental monitoring according to the approved follow-up schedule.

Standard rehabilitation in both groups

Standard rehabilitation in both groups was based on standardised protocols, with a fixed frequency of sessions and standardised exercise programmes; the intensity was adjusted according to the patients' tolerance and the severity of their neurological deficits. During the inpatient phase, sessions were held five times a week for 60–90 minutes, with additional daily short sessions of positioning and complication prevention lasting 15 to 20 minutes. The basic motor block included passive and actively assisted movements in the major joints (2–3 sets of 10 to 15 repetitions per segment) with a gradual transition to active exercises in the presence of voluntary contraction; Trunk control and balance in the sitting position were practised separately by holding the position for 30 to 60 seconds, with 5–8 repetitions, and weight shifting (2–3 sets of 8–12 repetitions). The functional block consisted of training in transferring in bed and repositioning, with 10 to 20 attempts per session, as well as training in self-care skills under the supervision of an occupational therapist, 3 to 5 times a week for 30 to 45 minutes.

Verticalisation was conducted in stages using an inclined table or standing frame, starting with 10–15 minutes and increasing to 30 minutes per session, whilst monitoring the blood pressure, heart rate and symptoms of orthostatic intolerance. Where indicated, functional electrical stimulation was used, specifically, the RT300 functional electrical stimulation (FES) exercise bike (*Restorative Therapies*, USA), usually 2 to 3 times a week for 20–30 minutes. Gait training, provided that there was sufficient neurological reserve, was conducted on a treadmill with partial body weight support, specifically, the *Woodway Loko Station* (*Woodway*, USA), or on the *Hausmann* Height and Width Adjustable Parallel Bars (*Hausmann Industries*, USA), 2 to 4 times a week, with a working interval

of 10–20 minutes and breaks as tolerated. In addition, ankle-foot or knee-level orthoses were used, such as the *Ottobock WalkOn AFO* (Germany), and walking aids such as a rollator, underarm crutches, a walking stick or a wheelchair were used, depending on the level of support required by the individual patient.

Prevention of contractures and management of spasticity were achieved through positioning, prolonged stretching and range-of-motion exercises, with stretching of the target muscle groups performed at least twice daily, holding each stretch for 30–60 seconds and repeating 3–5 times. Prevention of pressure ulcers was ensured by regularly changing the patient's position at intervals of approximately 2 hours whilst lying down, training in weight-bearing in a wheelchair, and the use of anti-decubitus mattresses and pillows. All sessions were documented in a rehabilitation chart, noting the duration of sessions, modules performed, tolerability, need for assistance, and functional changes achieved, to accurately correlate the intensity of rehabilitation with EMG, DTI MRI, and biomarker readings.

Monitoring and methods of evaluating results

Monitoring was conducted at fixed time points: T0 (baseline, prior to the start of the intervention), T1 (4 weeks), T2 (8 weeks), T3 (12 weeks), T4 (16 weeks), T5 (20 weeks), and T6 (24 weeks). At each time point, a clinical neurological assessment was performed, recording motor and sensory deficits according to American Spinal Injury Association (ASIA) [11], assessing muscle tone using the modified Ashworth scale [22], and determining functional independence by using the Spinal Cord Independence Measure (SCIM) [23], and the data were recorded in standardised protocols for subsequent comparison over time. Pelvic organ function was assessed by using item 6 of the SCIM (Sphincter Management – Bladder) at each assessment point; for an additional summary presentation, the proportion of patients showing improvement by T6 was calculated as a transition to a higher category of item 6.

The electrophysiological assessment included electromyography (needle or surface) and nerve conduction studies, which were performed at T0, T1, T2, T3, T4, T5 and T6. *Compound Muscle Action Potential* (CMAP) and distal motor latency, motor conduction velocity, and delayed motor response (F-wave) latency were recorded as an indicator of proximal conduction. Needle EMG data were used to assess the characteristics of motor unit action potentials, specifically, the *Motor Unit Action Potential* (MUAP) amplitude, and the presence of spontaneous activity in muscles below the level of injury. The primary comparisons assessed changes in each parameter relative to T0 within the group and the difference in changes between the groups at 12 and 24 weeks.

Neuroimaging monitoring was performed by using DTI-MRI according to a standardised scanning protocol at time points T0, T3, and T6. To quantitatively assess the integrity and organisation of the tracts, DTI parameters were analysed in the lesion area and in the adjacent segments above and below the level of injury. *Fractional Anisotropy* (FA) and *Mean Diffusivity* (MD) were used as indicators of changes in the tracts, as well as *Radial Diffusivity* (RD) and axial diffusivity in cases where these parameters could be correctly calculated within the defined analysis area in the lesion zone and adjacent segments. Additionally, provided the calculation was technically sound, radial and axial diffusivity were determined not only in the lesion zone but also in the adjacent segments above and below the level of injury. Changes from T0 to T3 and from T0 to T6 were compared within the group, as well as the difference in these changes between the main and the control groups.

The laboratory component involved the analysis of cerebrospinal fluid (CSF) obtained via lumbar puncture, with the determination of BDNF, NT-3, GAP-43, MAP-2 and GFAP at time points T0, T3 and T6. Measurements were performed by using quantitative solid-phase enzyme-linked immunosorbent assay (ELISA) with two antibodies against the respective marker, with

concentrations calculated by using a standard calibration curve. Concentrations were reported in pg/mL for BDNF and NT-3 and in ng/mL for GAP-43, MAP-2 and GFAP. The concentrations of each marker were compared relative to T0. To analyse the changes, Δ was calculated as the difference between the value at T6 and the value at T0 (T6–T0). The consistency of changes in biomarkers with the dynamics of EMG and DTI parameters at the same time points was then assessed.

Three time points were selected for the main presentation of results: baseline, 12 weeks and 24 weeks, as these corresponded to the baseline condition before intervention, an interim assessment following the completion of the early phase of the protocol, and a final assessment after six months of the follow-up, whilst interim visits were used primarily to monitor efficacy and safety.

Safety and control of undesirable phenomena

Throughout the study, adverse events were recorded at each follow-up visit and on a daily basis during the inpatient phase, by using standardised forms, with classification by severity and assessment of causality in relation to the intervention. Safety monitoring included clinical examinations, monitoring of vital signs and neurological status, as well as laboratory markers of systemic inflammatory response (complete blood count, C-reactive protein and basic biochemistry; coagulogram and procalcitonin wherever indicated). Complications of invasive procedures, particularly following lumbar puncture and local administration, were monitored separately; where neuroinflammation or infectious complications were suspected, additional investigations and management adjustments were carried out. Participation in the study or performance of the intervention was discontinued in the event of a significant adverse event, signs of a severe immune reaction, progressive neurological deterioration, or at the patient's request; serious events were reported to the principal investigator and the ethics committee in accordance with the institution's procedures.

Statistical analysis

Quantitative data were presented as *mean value±standard deviation* (M±SD). Changes within the groups were assessed by comparing the baseline values with specified reference points, whilst between-group differences were determined by analysing the change relative to the baseline. To assess the relationship between the biomarkers and instrumental parameters, a correlation analysis was performed between the changes (ΔT0–T6) in biomarkers and the corresponding changes in the EMG and DTI parameters, with the correlation coefficient r reported. A p-value of less than 0.05 was considered statistically significant.

Ethical principles

The study was conducted following the ethical principles of the World Medical Association's Declaration of Helsinki [24] and the requirements of Good Clinical Practice [25]. The Ethics Commission of the University of Medicine and Social Sciences, No. 444432, approved the study. Before inclusion, each participant was provided with full information regarding the aim, planned procedures, potential risks and expected benefits, following which, written informed consent was obtained. Confidentiality was ensured through data coding and restricted access to documentation. The participants were able to withdraw from the study at any time without losing access to standard medical care.

Results

Clinical and functional recovery at 12 and 24 weeks

Throughout the observation period, positive clinical progress was recorded in both groups; however, in the AXONIC-REVIVE™ group, this progress was more pronounced across a number of

indicators. The most notable differences concerned the motor component and the level of functional independence, which improved more rapidly and reached higher values at 12 and 24 weeks. In parallel, a more favourable trend was observed regarding the control of the muscle tone and pelvic functions, which was consistent with an improved daily activity in the patients at the end of the observation period. To summarise the key clinical and functional changes at the three assessment points, summary data are presented in Table 1, which shows the values at the baseline, at 12 and 24 weeks, as well as the magnitude of change from the baseline in each group (Table 1).

Table 1. Clinical and functional parameters over time and between-group differences

Indicator	Group	T0 M±SD	T3 M±SD	T6 M±SD	Change in T0-T6, M±SD	*p* between groups for change in T0-T6
ASIA Motor Score (0-100)	Main	48.2±11.6	58.9±12.1	66.8±12.9	+18.6±9.4	0.018
	Control	47.5±10.9	54.1±11.7	58.7±12.2	+11.2±8.7	
ASIA Sensory Score (0-224)	Main	142.7±22.5	154.8±23.1	162.4±24.0	+19.7±14.2	0.041
	Control	143.9±21.8	150.2±22.4	154.9±22.9	+11.0±13.6	
Modified Ashworth Scale (0-4), mean score	Main	2.1±0.6	1.6±0.6	1.3±0.5	-0.8±0.6	0.029
	Control	2.0±0.6	1.8±0.6	1.7±0.6	-0.3±0.6	
SCIM, total score (0-100)	Main	37.9±12.7	49.8±13.6	58.6±14.1	+20.7±10.8	0.012
	Control	38.6±12.1	45.1±13.0	50.7±13.5	+12.1±9.9	
Bladder control, with improvement to T6	Main	-	-	57.1% (16/28)	-	0.045
	Control	-	-	32.1% (9/28)	-	

Source: calculated by the author based on clinical observation data using the scales [22;24].

The data in Table 1 show that the ASIA motor score was one of the most sensitive indicators of intergroup differences. In the intervention group, the mean increase from T0 to T6 was +18.6 points, whereas, in the control group, it was +11.2 points, and the difference in change was statistically significant (p=0.018). The difference between the groups was already apparent by week 12: in the main group, the mean score rose to 58.9, and in the control group to 54.1. This early advantage has a practical explanation, as better motor control facilitates active training, standing up and practising transitions, and this enhances the effect of rehabilitation through a greater number of high-quality repetitions of motor actions. The sensory component also improved in both groups, but the increase was greater in the main group: +19.7 versus +11.0 points (p=0.041). Sensory changes are typically more variable; it is crucial that, at 24 weeks, the difference between the mean values became noticeable: 162.4 in the main group versus 154.9 in the control group. This indicates better integration of sensitivity into motor control, particularly during the stage of developing more stable functional skills.

Spasticity, as measured by the modified Ashworth scale, decreased in both groups, but the reduction was more pronounced in the treatment group: -0.8 versus -0.3 points (p=0.029). At the baseline, the groups were similar (2.1 and 2.0), and, by week 24, the mean score in the main group had fallen to 1.3, whilst, in the control group, it remained at 1.7. From a clinical perspective, lower spasticity means less resistance to passive and active movements, a lower risk of contracture formation, and greater effectiveness of strength and coordination exercises. Therefore, it is necessary to analyse this measure alongside functional scales, rather than in isolation. The SCIM score is the most relevant for everyday life. In the intervention group, the increase was +20.7 points, whereas, in the control group, it was +12.1 points; the difference in changes was significant (p=0.012). By week 12, the main group

already had a higher mean score (49.8 versus 45.1), and, by week 24, the difference had widened further (58.6 versus 50.7). This means that the changes were not limited to individual neurological scores, but reflected a real increase in independence, particularly in mobility, transferring, self-care and postural control. The bladder control score reflected changes in the pelvic function throughout the observation period. By week 24, improvement was recorded in 57.1% of the patients in the main group compared with 32.1% in the control group; the difference was significant (p=0.045). The data obtained indicate differences between the groups in the frequency of improvement in the pelvic functions, which may influence the need for assistance and independence in daily life.

Overall, the clinical and functional data are consistent: the AXONIC-REVIVE™ group showed greater improvement in the motor and sensory function, a more pronounced reduction in spasticity and higher levels of functional independence, as well as more frequent improvements in the bladder control. Together, these changes support the conclusion that there is a more favourable recovery trajectory up to 24 weeks compared with standard rehabilitation without experimental intervention.

Electrophysiological parameters of neuromuscular transmission and reinnervation

Electromyography and nerve conduction studies showed a gradual positive trend in both groups; however, in the AXONIC-REVIVE™ group, improvements began to emerge earlier and, by week 24, they had reached a more pronounced level for certain parameters. The most noticeable changes reflected the restoration of impulse conduction efficiency and an increase in the functional recruitment of motor units. At the week 12 interim stage, a trend towards greater increases in the motor response amplitude and improved speed characteristics was already evident in the main group, whereas, in the control group, such changes tended to accumulate more slowly and to a lesser extent. Table 2 presents the mean values and changes relative to the baseline, as well as an intergroup comparison of changes at T0, T3 and T6 (Table 2).

Table 2. Electrophysiological parameters over time and intergroup differences

Indicator	Group	T0 M±SD	T3 M±SD	T6 M±SD	Change in T0-T6 M±SD	*p* between groups for Change in T0-T6
CMAP amplitude, mW	Main	2.4±1.0	3.3±1.2	4.1±1.4	+1.7±1.0	0.021
	Control	2.5±1.1	2.9±1.1	3.3±1.3	+0.8±0.9	
Distal motor latency, ms	Main	6.2±1.3	5.6±1.2	5.1±1.1	-1.1±0.9	0.034
	Control	6.1±1.2	5.9±1.2	5.6±1.1	-0.5±0.8	
Motor conduction velocity, m/s	Main	38.7±7.5	42.9±7.8	46.1±8.2	+7.4±5.6	0.028
	Control	39.1±7.2	41.0±7.4	42.6±7.6	+3.5±5.1	
F-wave latency, ms	Main	61.4±8.6	58.2±8.2	55.9±7.9	-5.5±4.8	0.041
	Control	60.9±8.4	59.6±8.1	58.5±8.0	-2.4±4.6	
MUAP, amplitude, μV	Main	420±160	520±180	610±200	+190±150	0.019
	Control	430±170	470±170	520±180	+90±140	
Spontaneous activity, proportion of muscles showing fibrillation, %	Main	46.4	32.1	21.4	-25.0	0.046
	Control	44.6	38.4	32.1	-12.5	

Source: calculated by the author based on clinical observation data.

The data in Table 2 show that the CMAP amplitude increased in both groups, but the increase was greater in the main group. By week 24, the mean increase was +1.7 mV compared with +0.8 mV in

the control group (p=0.021). This trend is consistent with an increase in the number of functionally active motor units, as well as with greater synchrony in their recruitment during stimulation. By week 12, a difference between the groups was already apparent, indicating a faster change in the recovery trajectory in patients receiving multimodal intervention. Distal motor latency decreased in both groups, but the reduction was more pronounced in the control group (-1.1 ms versus -0.5 ms; p=0.034). Concurrently, motor conduction velocity showed a greater increase in the main group (+7.4 m/s versus +3.5 m/s; p=0.028). Together, these changes corresponded to improved impulse conduction along the peripheral pathway and a reduction in functional delays, which may be associated with demyelinating or compressive components of secondary changes. At week 12, the advantage of the main group was noticeable, but it was most evident by week 24, when the differences between the groups had accumulated.

The latency of the F wave decreased, which correlated with improved proximal conduction and more stable impulse conduction in the segmentally proximal regions. In the main group, the reduction was greater (-5.5 ms versus -2.4 ms; p=0.041), and, by week 12, a more favourable trajectory of change was emerging. Such dynamics may have reflected a reduction in functional delays in the reflex arc and an improved efficiency of motor neuron recruitment. Combined with an increase in conduction velocity and a reduction in distal latency, this supported the conclusion of a more complete recovery of neuromuscular transmission parameters.

Needle EMG at the motor unit level also differed between the groups. The MUAP amplitude increased in both groups, but the increase was greater in the control group (+190 μV versus +90 μV; p=0.019). This profile is consistent with the formation of more mature motor units during reinnervation and an increase in the contribution of collateral sprouting. Spontaneous activity warrants separate mention: the proportion of muscles exhibiting fibrillations decreased in both groups, but the reduction was more pronounced in the control group (-25.0% versus -12.5%; p=0.046). This reflected a tendency towards stabilisation of the state of denervated fibres and a reduction in activity, which usually accompanies acute denervation processes.

Overall, the electrophysiological data showed that the restoration of neuromuscular transmission and signs of reinnervation developed in both groups; however, in the AXONIC-REVIVE™ group, a faster rate of change and greater magnitude of improvement were observed up to week 24. The outcome profile was consistent with the control group's advantage in functional parameters, as an increased motor response amplitude, improved conduction, and reduced signs of denervation provided the electrophysiological basis for more stable performance of motor and daily living tasks.

Neuroimaging changes in the major pathways as revealed by diffusion tensor MRI

Diffusion tensor MRI data revealed changes in diffusion parameters in the injury zone and adjacent segments in both groups, which is consistent with a gradual restoration of the microstructural organisation of the nerve pathways over 24 weeks. At the same time, in the AXONIC-REVIVE™ group, the dynamics showed a greater amplitude, particularly in terms of indicators reflecting the orderliness of diffusion along the fibres and the degree of diffusion blurring in the lesion area. By week 12, a difference between the groups was already apparent in the injury zone, and, by week 24, the intergroup differences in changes became more pronounced, corresponding to the clinical and electrophysiological advantage of the main group.

For comparison between the groups, the values of fractional anisotropy and mean diffusivity are presented for the defined analysis region within the injury zone, as well as in the adjacent segments above and below the level of injury at points T0, T3, and T6. Additionally, for the injury zone, radial and axial diffusivity are presented as parameters that are more sensitive to changes in the transverse and longitudinal directions of diffusion (Table 3).

Table 3. Changes in DTI parameters in the injury site and adjacent segments

Indicator	Group	T0 M±SD	T3 M±SD	T6 M±SD	Change in T0-T6 M±SD	*p* between groups for change in T0-T6
Trauma area						
Fractional anisotropy	Main	0.29±0.06	0.34±0.06	0.38±0.07	+0.09±0.06	0.017
	Control	0.30±0.06	0.32±0.06	0.34±0.06	+0.04±0.05	
Average diffusivity, $\times10^{-3}$ mm²/s	Main	1.42±0.18	1.32±0.17	1.24±0.16	-0.18±0.15	0.023
	Control	1.40±0.17	1.35±0.17	1.32±0.16	-0.08±0.14	
Radial diffusivity, $\times10^{-3}$ mm²/s	Main	1.08±0.16	0.99±0.15	0.92±0.14	-0.16±0.13	0.019
	Control	1.06±0.15	1.02±0.15	0.99±0.15	-0.07±0.12	
Axial diffusivity, $\times10^{-3}$ mm²/s	Main	1.76±0.20	1.74±0.19	1.73±0.19	-0.03±0.15	0.312
	Control	1.75±0.19	1.73±0.19	1.72±0.18	-0.03±0.15	
Segment above injury						
Fractional anisotropy	Main	0.50±0.05	0.52±0.05	0.54±0.06	+0.04±0.04	0.046
	Control	0.50±0.05	0.51±0.05	0.52±0.05	+0.02±0.04	
Average diffusivity, $\times10^{-3}$ mm²/s	Main	0.98±0.10	0.96±0.10	0.94±0.10	-0.04±0.08	0.089
	Control	0.98±0.10	0.97±0.10	0.96±0.10	-0.02±0.08	
Segment below the injury						
Fractional anisotropy	Main	0.48±0.06	0.50±0.06	0.52±0.06	+0.04±0.05	0.038
	Control	0.48±0.06	0.49±0.06	0.50±0.06	+0.02±0.05	
Average diffusivity, $\times10^{-3}$ mm²/s	Main	1.02±0.11	0.99±0.11	0.97±0.10	-0.05±0.09	0.071
	Control	1.01±0.11	1.00±0.11	0.99±0.11	-0.02±0.09	

Source: calculated by the author based on clinical observation data.

Analysis of the data revealed that the most pronounced differences between the groups were observed at the injury site. Fractional anisotropy increased by +0.09 in the main group, compared with +0.04 in the control group, and the difference in these changes was statistically significant (p=0.017). Changes were already noticeable at week 12, when FA in the injury zone reached 0.34 in the main group compared with 0.32 in the control group, and, by week 24, the gap had widened (0.38 versus 0.34). An increase in FA in the lesion area is usually associated with a more ordered direction of diffusion along the fibres; therefore, this trend is consistent with the assumption of better microstructural organisation of the conduction pathways in the experimental group.

The mean diffusivity in the injury zone decreased in both groups, but the reduction was greater in the main group (-0.18 versus -0.08; p=0.023). These findings indicated that, by week 24, the main group exhibited a lower level of free diffusion in the affected tissue compared with the control group, which may correspond to a reduced oedematous component, a greater tissue density, or a more stable microstructure. Radial diffusivity also decreased more markedly in the main group (-0.16 versus -0.07; p=0.019). As RD is often regarded as a sensitive indicator of transverse changes in diffusion, its reduction could reflect more favourable dynamics of microstructural barriers in the transverse direction, consistent with the restoration of sheaths or a reduction in fibre disorganisation. In contrast, axial diffusivity showed minimal changes and did not differ between the groups over the T0–T6 period (p=0.312), thereby indicating a more stable nature of this parameter within the selected region of analysis throughout the observation period.

In the adjacent segments above and below the level of injury, the changes were less pronounced, but the trend in the main group remained more favourable. In the segment above the injury, FA increased by +0.04 in the main group compared with +0.02 in the control group (p=0.046). A similar

pattern was observed in the segment below the injury: +0.04 versus +0.02 (p=0.038). Such shifts in adjacent segments may reflect not only local changes but also the reorganisation of conduction pathways within the functional network, in particular due to the plasticity and adaptation of pathways to a new level of conductivity. The mean diffusivity in the adjacent segments decreased moderately; however, intergroup differences for this parameter did not reach statistical significance (p=0.089 and p=0.071), which corresponded to a weaker amplitude of changes outside the main lesion zone.

Overall, DTI findings confirmed a positive trend in the microstructural characteristics of the conduction pathways over 24 weeks in both groups; however, within the injury zone, the control group showed a greater increase in FA and a more pronounced decrease in MD and RD, reflecting more favourable changes within the affected area. Together with data from the clinical and electrophysiological studies, this correlates with a more pronounced trajectory of neural recovery in patients who received AXONIC-REVIVE™ compared with standard rehabilitation.

Cerebrospinal fluid biomarkers and their correlation with imaging data

Changes in cerebrospinal fluid biomarkers over 24 weeks showed that biological changes following injury occurred in both groups; however, in the AXONIC-REVIVE™ group, these changes exhibited a more pronounced profile in terms of markers of neurotrophic support and plasticity. As early as at week 12, differences between the groups were observed for some indicators, and, by week 24, these differences had become more pronounced. Changes in the cerebrospinal fluid did not occur in isolation but correlated with instrumental indicators of recovery, in particular, with electrophysiological parameters and diffusion MRI indices. This supported consistency between the biological response and functional recovery within the specified efficacy criteria. Before presenting the correlations, the concentrations of BDNF, NT-3, GAP-43, MAP-2 and GFAP at the baseline, at 12 and 24 weeks, as well as the change relative to the baseline in each group, are shown. The summarised data are presented in Table 4.

Table 4. Changes in cerebrospinal fluid biomarker concentrations over time and differences between groups

Indicator, units	Group	T0 M±SD	T3 M±SD	T6 M±SD	Change in T0-T6 M±SD	*p* between groups for a change in T0-T6
BDNF, pg/mL	Main	24.8±8.1	33.9±9.4	38.7±10.2	+13.9±9.0	0.018
	Control	25.3±8.0	29.1±8.7	31.6±9.1	+6.3±8.6	
NT 3, pg/mL	Main	18.9±6.2	25.6±7.1	29.8±7.9	+10.9±7.0	0.022
	Control	19.2±6.0	22.1±6.5	23.9±6.8	+4.7±6.6	
GAP 43*, ng/mL	Main	1.62±0.52	2.06±0.61	2.28±0.66	+0.66±0.55	0.029
	Control	1.65±0.50	1.85±0.56	1.96±0.59	+0.31±0.51	
MAP 2* , ng/mL	Main	3.10±0.92	3.78±1.03	4.16±1.10	+1.06±0.88	0.031
	Control	3.06±0.90	3.42±0.96	3.60±1.01	+0.54±0.84	
GFAP, ng/mL	Main	2.35±0.71	2.02±0.66	1.78±0.60	-0.57±0.55	0.040
	Control	2.32±0.70	2.18±0.68	2.08±0.64	-0.24±0.52	

Note: GAP 43* – growth-associated protein 43; MAP 2* – Microtubule-associated protein 2.
Source: calculated by the author based on clinical observation data.

The first set of results concerns the neurotrophins BDNF and NT-3, which were considered in the study as markers of neurotrophic support and conditions conducive to plasticity. The baseline values in both groups were similar, thus making subsequent differences easier to interpret. By week

12, BDNF in the main group had risen to 33.9 pg/mL, whilst, in the control group, it had risen to 29.1 pg/mL; by week 24, the gap persisted and became more pronounced (38.7 versus 31.6 pg/mL). The difference between the groups was evident not only in the end-point values but also in the magnitude of the increase from T0 to T6: +13.9 in the main group versus +6.3 in the control group (p=0.018). A similar pattern was observed for NT 3, where the increase was +10.9 versus +4.7 (p=0.022). This profile indicates that the increase in neurotrophins in the intervention group was not a one-off event, but, rather, it formed a more sustained trajectory up to 24 weeks. When compared with clinical and electrophysiological dynamics, the early rise in neurotrophins has a positive effect on the differences already observed at week 12.

GAP 43 is more commonly associated with growth and plasticity processes in the neural tissue, whereas MAP 2 reflects the state of the structural components of neurons and may change as neuronal organisation stabilises. Table 4 shows that both markers increased in both groups, but the increase was greater in the main group. For GAP 43, the change from T0 to T6 was +0.66 ng/mL versus +0.31 ng/mL in the control group (p=0.029), and, for MAP 2, +1.06 versus +0.54 ng/mL, respectively (p=0.031). By week 12, the difference was already noticeable, but it was most pronounced at week 24, when the mean values in the main group reached 2.28 ng/mL for GAP 43 and 4.16 ng/mL for MAP 2. This supports the hypothesis that the conditions for remodelling and plasticity are established throughout the entire observation period, rather than just in the initial few weeks.

Table 4 shows a decline in the GFAP levels in both groups, which is consistent with the gradual resolution of the acute phase. However, the reduction was more pronounced in the control group: -0.57 ng/mL versus -0.24 ng/mL (p=0.040). At week 12, the difference was not yet marked (2.02 versus 2.18 ng/mL), but, by week 24, it had become more noticeable (1.78 versus 2.08 ng/mL). Such changes do not indicate the disappearance of the glial response, but, rather, its less intense persistence in the later phase, which may create more favourable conditions for plastic changes. To assess the extent to which biomarker changes correlate with instrumental findings, a correlation analysis was performed on biomarker increases from T0 to T6 with key electrophysiological and DTI parameters (Table 5).

Table 5. Correlations between changes in biomarkers and EMG and DTI parameters (T0–T6)

Biomarker change (T0-T6)	ΔCMAP, r	Conductivity rate, r	ΔFA in trauma area, r
ΔBDNF	0.46	0.41	0.43
3 ΔNT	0.39	0.37	0.40
43 ΔGAP	0.44	0.35	0.47
2 ΔMAP	0.33	0.29	0.36
ΔGFAP	-0.31	-0.27	-0.34

Source: calculated by the author based on clinical observation data.

The data in Table 5 show that increased BDNF levels were associated with a greater increase in the CMAP amplitude (r=0.46) and an increase in FA in the injury zone (r=0.43). This is explained by the fact that higher levels of neurotrophic support are more often accompanied by better functional activity of motor units and more organised microstructural characteristics of the conduction pathways. NT 3 demonstrated a similar, albeit somewhat weaker, correlation profile, indicating a common direction of neurotrophin changes in the recovery processes. GAP 43 had one of the strongest correlations with ΔFA (r=0.47) and a notable correlation with ΔCMAP (r=0.44). This may imply that a greater increase in the plasticity marker was more frequently accompanied by better microstructural changes and a stronger electrophysiological response. For MAP 2, the correlations were weaker, which seemed to be expected for a marker that may respond more slowly

– and not always linearly – particularly in the context of individually varying recovery trajectories. Negative correlations between GFAP and ΔCMAP, conduction velocity, and ΔFA (ranging from -0.27 to -0.34) indicated that a more pronounced reduction in the glial activity was more frequently associated with better functional outcomes. At the same time, the correlation coefficients remained moderate, suggesting that the biomarkers reflected only one aspect of recovery rather than the entire mechanism of the effect. Such findings are to be expected, as functional outcomes are influenced by multiple processes, and very high correlations are rarely observed in real-world clinical data.

Thus, analysis of cerebrospinal fluid biomarkers revealed that, in the AXONIC-REVIVE™ group, increases in BDNF, NT-3, GAP-43 and MAP-2 were more pronounced, and the reduction in GFAP was more marked by week 24, compared with the control group. Moderate correlations between changes in biomarkers and ΔCMAP, conduction velocity and ΔFA demonstrated a link between biological changes and instrumental signs of recovery and complemented the clinical findings.

Discussion

Over the 24-week observation period, positive trends were observed in both groups; however, in the AXONIC-REVIVE™ group, improvements in several parameters were greater. At 12 and 24 weeks, this group recorded higher scores for the motor and sensory components of the ASIA scale, a more pronounced reduction in spasticity on the modified Ashworth scale, and a greater improvement in functional independence on the SCIM. The proportion of patients with an improved bladder control up to T6 was also higher in the intervention group. Cerebrospinal fluid biomarkers showed greater changes in BDNF, NT-3, GAP-43 and MAP-2, and a more pronounced reduction in GFAP, whilst correlations with EMG and DTI indicated a link between biological changes and instrumental measures of recovery.

In the clinical arm, by week 24, the main group showed a greater improvement in the ASIA Motor Score (+18.6 versus +11.2; p=0.018) and the SCIM (+20.7 versus +12.1; p=0.012), as well as a more pronounced reduction in spasticity on the modified Ashworth scale (-0.8 versus -0.3; p=0.029) and a higher proportion of improvement in the bladder control (57.1% versus 32.1%; p=0.045). In a conceptual review, the multi-level nature of recovery assessment was described such that clinical scores had to be corroborated by functional outcomes, as noted by M. Tuszynski and O. Steward [26]. The greater increase in the SCIM scores, together with the increase in the ASIA Motor scores, was consistent with the reasoning, as the changes affected both the neurological scores and functional independence. J. Kaniuk et al. [27] summarised the mechanisms of remyelination as a prerequisite for the restoration of conduction velocity and motor coordination. The more pronounced dynamics of motor recovery and reduction in spasticity found do not contradict this explanation, although a direct assessment of remyelination was not performed in this study, and the conclusion thus remained indirect.

In a meta-analysis by J. Hirt et al. [28], the results of experimental interventions aimed at inhibiting the proteins of the Nogo family (myelin-associated axon growth inhibitors) (Nogo) pathway were synthesised, demonstrating that functional improvements occurred frequently, but their magnitude varied depending on the injury model, the timing of administration, and endpoints. Greater gains in the control group on the ASIA Motor and SCIM scales were consistent with the general direction of these findings; however, the distribution of the effect across the components of the protocol could not be disentangled in this design. In the study by S. Yadav and S. Mukherjee [29], secondary injury following SCI was described as a cascade of inflammation, glial reaction and inhibitory signals, which limited recovery. The more pronounced dynamics in the main group, in particular, a greater reduction in spasticity and greater sensory improvement, were consistent with the presented model

of multi-level effects. T. Zhai et al. [30] linked training following SCI to the activation of plasticity and recovery processes. The higher early motor scores at 12 weeks (58.9 versus 54.1) may have corresponded to this mechanism, but the standardised rehabilitation in both groups meant that the between-group difference could not be explained by the training factor alone.

Electrophysiological data showed a more favourable trend in the main group for key indicators of conduction and reinnervation: the CMAP amplitude increased by +1.7 mV compared with +0.8 mV, the motor conduction velocity increased by +7.4 compared with +3.5 m/s, and the F-wave latency decreased by -5.5 versus -2.4 ms, with intergroup differences at a *p*-value of less than 0.05. In the experiment conducted by A. Fournier et al. [31], ROCK inhibition attenuated the neuronal response to myelin inhibitors and promoted axonal outgrowth; therefore, the greater increases in CMAP and conduction velocity appeared consistent with a model in which the structural and functional pathways of recovery were facilitated. In a systematic review by A. Khavandegar et al. [32], it was shown that RhoA or ROCK inhibition most often yielded better functional outcomes when initiated early and administered at the correct dosage regimen. The differences observed in this study at 12 weeks are consistent with the authors' findings, as a more rapid improvement in electrophysiological parameters typically reflects a more favourable course of recovery.

A study by E. Bradbury et al. [33] demonstrated that the enzymatic degradation of CSPG in glial scar tissue using ABC chondroitinase was accompanied by significant improvements in functional recovery following SCI. The reduction in denervation signs and improved conduction parameters in the experiment were consistent with the finding that the removal of matrix barriers facilitated conditions for reinnervation and supported recovery processes. In a review by M. Schwab [34], it is described that Nogo signals formed an inhibitory microenvironment that suppressed axon growth and plasticity following central nervous system (CNS) injury. The positive dynamics of MUAP and the reduction in spontaneous activity in this study corresponded to the expected direction of changes under conditions of reduced inhibitory influences and the gradual restoration of neuromuscular interaction. However, M. Simis et al. [35] emphasised that neurophysiological markers reflected different levels of the nervous system, and the strength of associations with function might be incomplete; therefore, the interpretation of EMG indicators must remain multifaceted. In particular, an increase in CMAP did not necessarily indicate full restoration of the central conduction but could also reflect peripheral reinnervation mechanisms and training effects.

DTI results showed that, in the injury zone of the main group, the increase in FA was greater (+0.09 versus +0.04; p=0.017), and the reduction in MD and RD was more pronounced (-0.18 versus -0.08 and -0.16 versus -0.07×10^{-3} mm²/s; p<0.05), whilst AD did not differ significantly. T. Feng et al. [36] considered DTI metrics as indicators of long-term changes in neural networks following SCI, with the correlation with clinical dynamics being most evident when the analysis was performed in anatomically relevant regions. Therefore, a greater increase in FA in the injury zone in the main group, together with a more pronounced decrease in MD, appeared consistent with the researchers' approach, where the signal most often manifested where structural changes were expected. An experiment by Z. Wen et al. [37] showed that a laminin-like environment formed a microarchitecture conducive to the directed growth and stabilisation of processes. The combination of an upward trend in FA and a downward trend in RD in the main group (+0.09 and -0.16) was consistent with the idea of a more ordered microstructure, as RD responds to changes associated with the transverse looseness of the tissue.

F. Fang et al. [38] emphasised that collagen-based materials provide not only mechanical support but also a substrate for cell adhesion and local bioactivity, which promotes remodelling. The more pronounced DTI profile of changes in the injury zone in the main group was consistent with this description, as the collagen-laminin hydrogel in the protocol functioned precisely as a local matrix,

where changes were expected to accumulate more rapidly than in distant segments. In the study by O. Rybachuk et al. [39], it was emphasised that combining a matrix with biological agents yields the greatest effect when the delivery time is correctly selected, and the exposure duration is sufficient. The fact that differences in DTI parameters were noticeable at 12 weeks and persisted until 24 weeks was consistent with the scientists' conclusion regarding the medium-term window when remodelling becomes measurable. S. Wei et al. [40] summarised that MSCs and their derivatives act primarily through paracrine signalling and modulation of the microenvironment, rather than solely through tissue replacement. Therefore, the more pronounced DTI changes in the main group can be attributed to this mechanism: part of the effect may have been due to biological support of remodelling, rather than solely through structural support of the matrix.

Cerebrospinal fluid biomarkers up to 24 weeks showed greater changes in the main group: the increase in BDNF was +13.9 versus +6.3 pg/mL (p=0.018), NT-3 was +10.9 versus +4.7 pg/mL (p=0.022), whilst GFAP decreased by -0.57 versus -0.24 ng/mL (p=0.040). Plasticity markers also increased: GAP-43 by +0.66 versus +0.31 ng/mL (p=0.029) and MAP-2 by +1.06 versus +0.54 ng/mL (p=0.031); correlations revealed a positive association between ΔBDNF and increases in CMAP (r=0.46) and ΔFA (r=0.43), whilst negative correlations of up to -0.34 were observed for GFAP. That BDNF modulates the plasticity and remodelling of synaptic connections was confirmed by C. Toader et al. [41], but the direction of the effect depended on the microenvironmental context and the balance between adaptive and undesirable remodelling. Therefore, the increase in BDNF in this study should be interpreted as a marker of activated recovery processes, and the moderate correlations with ΔFA and CMAP supported the interpretation via plasticity rather than as an automatic guarantee of a functional outcome. Mechanistic evidence that BDNF can direct the growth and targeted integration of sprouts following SCI was presented in Y. Li et al. [42]. In this context, the association of ΔBDNF with ΔFA and CMAP appeared consistent with a model of trophically-driven remodelling, where the biological signal is reflected in both the microstructure of the tracts and the electrophysiological response.

The role of NT-3 in supporting recovery and influencing spasticity is described in Z. Talifu et al. [43], where neurotrophin was linked to changes relevant to motor control. The greater increase in NT 3 in the main group, together with a greater reduction in the muscle tone according to the Ashworth scale (-0.8 versus -0.3), is consistent with this logic and explains why changes in biomarkers could be reflected in clinical indicators. The synergy of ABC chondroitinase with other approaches in combination regimens was summarised by R. Cunha et al. [44], emphasising that the effect was enhanced precisely when matrix remodelling was combined with trophic or cellular signals. The profile showing greater increases in GAP 43 and MAP 2 in the context of a greater reduction in GFAP in the control group was consistent with the concept of complex environmental modulation, where barriers were simultaneously weakened, and markers of structural remodelling were activated. The issue of enzyme stability and duration of action was examined in detail in a paper by S. Koushki et al. [45], which emphasised the role of delivery conditions and the local bioavailability for the reproducibility of effects. This explains why biomarker dynamics at 12 and 24 weeks could be shaped not only by biological mechanisms but also by how long the components remained active in the area of influence, whilst moderate correlations indicated that the recovery was multifactorial.

The results obtained are consistent with those of previous studies, which highlighted the benefits of combining multiple targets to overcome the inhibitory environment following SCI. More pronounced improvements in the main group, as measured by clinical scales, electrophysiological parameters and DTI metrics, support the approach described in studies on the Nogo pathway blockade, RhoA or ROCK inhibition, and matrix remodelling via chondroitinase ABC. The dynamics of neurotrophins and plasticity markers also correspond to studies linking BDNF and NT-3 to processes of recovery and network reorganisation in the subacute and medium-term periods.

Conclusions

The study results showed that by, week 24, the main group had demonstrated a more pronounced recovery in the motor function and activities of daily living compared with the control group. The increase in the ASIA Motor Score was +18.6 points versus +11.2, and the increase in the SCIM was +20.7 versus +12.1. These differences indicate that the changes affected not only the neurological scores but also practical skills related to mobility, self-care and the performance of basic daily activities. Electromyography and conduction studies showed a faster positive trajectory in the intervention group at the key assessment points of 12 and 24 weeks. The reduction in F-wave latency was greater in the intervention group, -5.5 ms versus -2.4 ms (p=0.041), indicating better dynamics of proximal conduction and segmental integration of the motor pathway. Together with trends towards an increased motor response and a reduced denervation activity, this supported the interpretation of more active reinnervation processes.

DTI parameters in the injury zone revealed more pronounced remodelling of the white matter tracts in the main group. The increase in fractional anisotropy was +0.09 versus +0.04 (p=0.017), and the reduction in mean and radial diffusivity was more pronounced than in the control group. This combination of changes indicated a more organised tissue microstructure in the lesion area in the medium term, whilst the absence of a clear difference in axial diffusivity suggests that the indicators were not equally sensitive at this stage. By week 24, greater shifts in neurotrophins and markers of plasticity were recorded in the main group, reflecting more active biological support for recovery. The increase in BDNF was +13.9 versus +6.3 pg/mL, whilst GFAP decreased by -0.57 versus -0.24 ng/mL. Correlations showed a link between ΔBDNF and the increase in CMAP (r=0.46) and ΔFA (r=0.43), indicating parallel biological and instrumental changes in some patients and supporting the notion of a multi-component nature of recovery.

A limitation of the study is the relatively short follow-up period (up to 24 weeks), which means that the long-term durability of clinical, DTI and cerebrospinal fluid changes remains unconfirmed. Consequently, the present findings cannot determine whether AXONIC-REVIVE™ improves the final extent of neural recovery or primarily accelerates recovery during the initial 24 weeks, as the outcomes of the two groups may converge during a longer-term follow-up. In future studies, it would be advisable to extend the follow-up to at least 12 months with fixed time points to confirm the duration of the effect, and to compare it more accurately with instrumental and biomarker trajectories.

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Author contributions

The author is solely responsible for the conceptualization, methodology, investigation, data analysis, writing of the original draft, review and editing of the manuscript. The author has approved the final version for publication.

Ethics Approval

Ethics Commission of the University of Medicine and Social Sciences, No. 444432, approved the study.

List of abbreviations:

AAV9 – adeno-associated virus serotype 9;
ABC – chondroitinase ABC;
ASIA – American Spinal Injury Association;
BDNF – brain-derived neurotrophic factor;
CD133 – differentiation cluster 133;
CMAP – compound muscle action potential;
CNS – central nervous system;
DTI – diffusion tensor imaging;
DTI-MPT – diffusion tensor magnetic resonance imaging;
EMG – electromyography;
FA – fractional anisotropy;
FES – functional electrical stimulation;
F-wave – delayed motor response;
GAP 43 – growth-associated protein 43;
GFAP – glial fibrillary acidic protein;
M±SD – mean value ± standard deviation;
MAP 2 – microtubule-associated protein 2;
MD – mean diffusivity;
MSC – mesenchymal stem cells;
MUAP – motor unit action potential;
Nogo – proteins of the Nogo family (myelin-associated axon growth inhibitors);
Nogo A– Nogo protein isoform;
PBS – phosphate-buffered saline;
RD – radial diffusivity;
Rho – family of small Rho GTPases (cytoskeletal regulators);
RhoA – RhoA, a small GTPase from the Rho family;
ROCK – Rho-associated coiled-coil containing protein kinase;
SCI – spinal cord injury;
SCIM – Spinal Cord Independence Measure.

References

1. World Health Organization. Spinal cord injury [Internet]. 2024. https://www.who.int/news-room/fact-sheets/detail/spinal-cord-injury
2. Ferrara UP, Tortora C, Rosano C, Assunto A, Rossi A, Pagano S, et al. Bone metabolism in patients with type 1 neurofibromatosis: key role of sun exposure and physical activity. *Sci Rep*. 2022;12(1):4368. doi:10.1038/s41598-022-07855-4
3. Zaika V, Tytarenko Ya. A review of the therapeutic and neuroprotective properties of ketamine. *Med Sci Ukr*. 2026;22(2):170-177. doi:10.32345/2664-4738.2.2026.18
4. Sagymbayev M, Koichubekov A, Rahmanov B, Nurzhan uulu U. Recovery treatment in the intermediate and late periods of spinal cord injury. *Eurasian Health J*. 2024;16(4):168-172. doi:10.54890/1694-8882-2024-4- 168
5. Głowacka A, Ji B, Szczepankiewicz AA, Skup M, Gajewska-Woźniak O. BDNF spinal overexpression after spinal cord injury partially protects soleus neuromuscular junction from disintegration, increasing VAChT and AChE transcripts in soleus but not tibialis anterior motoneurons. *Biomedicines*. 2022;10(11):2851. doi:10.3390/biomedicines10112851
6. Suszyński K, Białoń N, Marcol W, Górka D, Górka M. The influence of reinnervation on the results of peripheral nerve repair after injuries. *Advances in Hygiene and Experimental Medicine*. 2023;77(1):118-124. doi:10.2478/ahem-2023-0012
7. Nowacka A, Śniegocki M, Ziółkowska E. A review of the potential use of antioxidants in spinal cord injuries. *Antioxidants (Basel)*. 2025;14(9):1081. doi:10.3390/antiox14091081

8. Pedrini FA, Boriani F, Bolognesi F, Fazio N, Marchetti C, Baldini N. Cell-enhanced acellular nerve allografts for peripheral nerve reconstruction: A systematic review and a meta-analysis of the literature. *Neurosurgery*. 2019;85(5):575-604. doi:10.1093/neuros/nyy374
9. Serra PL, Boriani F, Khan U, Atzeni M, Figus A. Rate of free flap failure and return to the operating room in lower limb reconstruction: A systematic review. *J Clin Med*. 2024;13(15):4295. doi:10.3390/jcm13154295
10. Yrysov K, Tashibekov J, Baktybek uulu M, Kazyev A. Results of diagnosis and surgical treatment of spinal cord injury. *Eurasian Health J*. 2024;16(3):163-171. doi:10.54890/1694-8882-2024-3-163
11. Niemczyk-Soczynska B, Zaszczyńska A, Zabielski K, Sajkiewicz P. Hydrogel, electrospun and composite materials for bone/cartilage and neural tissue engineering. *Materials (Basel)*. 2021;14(22):6899. doi:10.3390/ma14226899
12. Basiaga B, Baczkowski S, Żądecka-Kobiałka K, Putra A, Marchaj M, Nowicka-Jasińska M, et al. The current state of stem cell therapy for spinal cord injury and other systemic injuries. *Archiv EuroMedica*. 2024;14(2). doi:10.35630/2024/14/2.207
13. Głowacka A. *Stimulation of neuronal overproduction of BDNF in the injured rat spinal cord: evaluation of molecular and morphological changes during the period of greatest functional improvement*. Doctoral dissertation. Nencki Institute of Experimental Biology PAS; 2023. http://rcin.org.pl/ibd/Content/241160/WA488_277578_20304_Glowacka-Anna-2023.pdf
14. Latka K, Kozlowska K, Waligora M, Kolodziej W, Latka D. Effect of DiscoGel treatment of the intervertebral disc at MRI. *Clin Radiol*. 2023;78(12):928-934. doi:10.1016/j.crad.2023.07.023
15. Moffa S, Perna A, Cattolico A, Sellitto C, Ascione A, Tafuri D, et al. Evaluations of muscular strength, ability to balance and health status in prisoners during covid-19. *Int J Envir Res Public Health*. 2021;18(8):4316. doi:10.3390/ijerph18084316
16. Efremov A. Integration of AI and Neuroscience: New Methods in Studying the Causes of Mental Disorders. *Int J Psychiatry Med*. 2026;61(4):498-514. doi:10.1177/00912174261428872
17. Khramtsov DM, Stoyanov O, Hruzevskiy O, Shevchuk HYu. Complex neurorehabilitation of post-stroke patients. *Med Sci Ukr*. 2022;18(3):52-59. doi:10.32345/2664-4738.3.2022.08
18. Zimny A, Podgórski P, Machaj W, Fortuna W, Huber J, Bobek-Billewicz B, et al. Diffusion tensor imaging findings in cerebral sensorimotor areas in patients after spinal cord injury correlate with neurophysiological deficits. *Neurorehabil Neural Repair*. 2025;39(9):701-714. doi:10.1177/15459683251345434
19. Huber J, Leszczyńska K, Wincek A, Szymankiewicz-Szukała A, Fortuna W, Okurowski S, et al. The role of peripheral nerve electrotherapy in functional recovery of muscle motor units in patients after incomplete spinal cord injury. *Appl Sci*. 2021;11(20):9764. doi:10.3390/app11209764
20. Waliullah S, Singh A, Rastogi D, Kumar D, Kumar A, Sharma AC, et al. Serum Nogo-A as a predictor of neurological recovery in spinal cord injury. *Bioinformation*. 2025;21(9):3387-3390. doi:10.6026/973206300213387
21. International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI/ASIA): worksheet and classification materials [Internet]. https://pdmu.edu.ua/storage/ft_nnmi_zo/docs/sHa8biST8guL62YaR89qbFK98GzuOLwTopXe35l9.pdf
22. Shirley Ryan AbilityLab. Modified Ashworth Scale (MAS) instructions [Internet]. 2026. https://www.sralab.org/sites/default/files/2017-06/Modified%20Ashworth%20Scale%20Instructions.pdf
23. Shirley Ryan AbilityLab. Spinal Cord Independence Measure (SCIM III) [Internet]. 2026. https://www.sralab.org/sites/default/files/2017-07/SCIM.pdf
24. World Medical Association. Declaration of Helsinki: ethical principles for medical research involving human subjects [Internet]. 2024. https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/
25. European Medicines Agency. ICH E6(R2) Good clinical practice: scientific guideline [Internet]. 2025. https://www.ema.europa.eu/en/ich-e6-good-clinical-practice-scientific-guideline
26. Tuszynski MH, Steward O. Concepts and methods for the study of axonal regeneration in the CNS. *Neuron*. 2012;74(5):777-791. doi:10.1016/j.neuron.2012.05.006
27. Kaniuk JK, Kumar D, Tennyson J, Hurka KL, Margolis A, Bucaloiu A, et al. Spinal cord injury remyelination: pathways to therapies. *Int J Mol Sci*. 2025;26(15):7249. doi:10.3390/ijms26157249
28. Hirt J, Khanteymoori A, Hohenhaus M, Kopp MA, Howells DW, Schwab JM, et al. Inhibition of the Nogo-pathway in experimental spinal cord injury: a meta-analysis of 76 experimental treatments. *Sci Rep*. 2023;13:22898. doi:10.1038/s41598-023-49260-5

29. Yadav S, Mukherjee S. Pathophysiology of spinal cord injury and advanced therapeutic approaches. *Mol Biol Rep*. 2025;52(1):669. doi:10.1007/s11033-025-10785-9
30. Zhai T, Ren S, Qian S, Shi C, Wang B, Zhang C, et al. Exercise training promotes nerve cell repair and regeneration after spinal cord injury. *Neural Regen Res*. 2026;21(6):2153-2168. doi:10.4103/nrr.nrr-d-24-01677
31. Fournier AE, Takizawa BT, Strittmatter SM. Rho kinase inhibition enhances axonal regeneration in the injured CNS. *J Neurosci*. 2003;23(4):1416-1423. doi:10.1523/JNEUROSCI.23-04-01416.2003
32. Khavandegar A, Ahmadi NS, Mousavi MA, Ramezani Z, Khodadoust E, Hasan Zadeh Tabatabaei MS, et al. The potential role of RhoA/ROCK-inhibition on locomotor recovery after spinal cord injury: a systematic review of in-vivo studies. *Spinal Cord*. 2025;63(3):95-126. doi:10.1038/s41393-025-01064-2
33. Bradbury EJ, Moon LD, Popat RJ, King VR, Bennett GS, Patel PN, et al. Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature*. 2002;416(6881):636-640. doi:10.1038/416636a
34. Schwab ME. Functions of Nogo proteins and their receptors in the nervous system. *Nat Rev Neurosci*. 2010;11(12):799-811. doi:10.1038/nrn2936
35. Simis M, Doruk Camsari D, Imamura M, Filippo TRM, Rubio De Souza D, Battistella LR, et al. Electroencephalography as a biomarker for functional recovery in spinal cord injury patients. *Front Hum Neurosci*. 2021;15:548558. doi:10.3389/fnhum.2021.548558
36. Feng T, Zhao C, Su WN, Gao YM, Wu YY, Zhao W, et al. Longitudinal changes in brain network metrics and their correlations with spinal cord diffusion tensor imaging parameters following spinal cord injury and regenerative therapy. *Biomedicines*. 2025;13(12):3124. doi:10.3390/biomedicines13123124
37. Wen Z, Song L, Liu P, Chin ATZE, Zhou W, Hu Q. A laminin-mimicking hydrogel as regenerative microenvironment for the repair of peripheral nerve injury. *Colloids Surf A Physicochem Eng Asp*. 2025;723:137369. doi:10.1016/j.colsurfa.2025.137369
38. Fang F, Wang X, Tao Y, Su Y, Dan W, Du Z, et al. Collagen-based biomaterials in neural injury repair: current advances and future perspectives. *Collagen Leather*. 2025;7(1):27. doi:10.1186/s42825-025-00209-6
39. Rybachuk O, Nesterenko Y, Zhovannyk V. Modern advances in spinal cord regeneration: hydrogel combined with neural stem cells. *Front Pharmacol*. 2024;15:1419797. doi:10.3389/fphar.2024.1419797
40. Wei S, Dong J, Hu Q, Bai J, Gao X, Shan H, et al. Advances in mesenchymal stem cells and their derivatives for promoting peripheral nerve regeneration. *Burns Trauma*. 2025;13:tkaf027. doi:10.1093/burnst/tkaf027
41. Toader C, Serban M, Munteanu O, Covache-Busuioc RA, Enyedi M, Ciurea AV, et al. From synaptic plasticity to Neurodegeneration: BDNF as a transformative target in medicine. *Int J Mol Sci*. 2025;26(9):4271. doi:10.3390/ijms26094271
42. Li Y, Tran A, Graham L, Brock J, Tuszynski MH, Lu P. BDNF guides neural stem cell-derived axons to ventral interneurons and motor neurons after spinal cord injury. *Exp Neurol*. 2023;359:114259. doi:10.1016/j.expneurol.2022.114259
43. Talifu Z, Qin C, Xin Z, Chen Y, Liu J, Dangol S, et al. The overexpression of insulin-like growth factor-1 and neurotrophin-3 promote functional recovery and alleviate spasticity after spinal cord injury. *Front Neurosci*. 2022;16:863793. doi:10.3389/fnins.2022.863793
44. Cunha RS, Rossi EA, Santana TAD, Costa-Ferro ZSM, Souza BSDF. Chondroitinase ABC in spinal cord injury: advances in delivery strategies and therapeutic synergies. *Front Bioeng Biotechnol*. 2025;13:1604502. doi:10.3389/fbioe.2025.1604502
45. Koushki S, Askaripour H, Bahiraee S. Review on strategies for enhancing stability of chondroitinase ABC in the treatment of spinal cord injury. *Discov Appl Sci*. 2025;7:219. doi:10.1007/s42452-025-06656-4