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Biochemical and Neuroimmunological Changes Associated with Motor Function Recovery in an Experimental Animal Model of Spinal Cord Injury

Nizom Ermatov[1], Ruxulla Xikmatullayev[2], Bakhtiyar Iriskulov[3] Bakhramjon Mamatkulov[4], Dilshod Alimukhamedov[5], Mukharrama Xasanova[6], Kamol Ibrohimov[7]*, Mavlonjon Nasirdinov[8]

[1] Doctor of Medical Sciences, Professor, Head of the Department of Hygiene of Children, Adolescents and Nutrition, Tashkent State Medical University, Tashkent, Uzbekistan.

[2] PhD, Department of Normal and Pathological Physiology, Tashkent State Medical University, Tashkent, Uzbekistan.

[3] Professor, Doctor of Medical Sciences, Head of the Department of Normal and Pathological Physiology, Tashkent State Medical University, Tashkent, Uzbekistan.

[4] Doctor of Medical Sciences, Professor, School of Public Health, Tashkent State Medical University, Tashkent, Uzbekistan.

[5] Professor, Doctor of Medical Sciences, Department of Hygiene of Children, Adolescents and Nutrition, Tashkent State Medical University, Tashkent, Uzbekistan.

[6] Doctor of Medical Sciences, Professor, Department of Forensic Medicine and Medical Law, Tashkent State Medical University, Tashkent, Uzbekistan.

[7] PhD, Department of Hygiene, Bukhara State Medical Institute named after Abu Ali ibn Sino, Bukhara, Uzbekistan. ikamol030@gmail.com

[8]PhD, Head of the Department of Physiology and Pharmacology, Central Asian Medical University, Fergana City P150105, Uzbekistan.

Abstract

Introduction: Spinal cord injury (SCI) triggers secondary neuroinflammatory and autoimmune cascades inducing autoantibody production against neural antigens, contributing to progressive neurological impairment. This study evaluated the dynamics of motor function and neurotropic autoantibody profiles following experimental SCI using the ELI-Neuro-Test.

Material and methods: Experimental spinal injury was induced in 98 adult male outbred rats using a standardized weight-drop model. Voluntary locomotor activity, hindlimb grip strength, and motor coordination were assessed on days 3, 7, and 14 post-injury. Serum IgG autoantibodies against 12 targets were measured using the ELI-Neuro-Test and analyzed via Spearman's correlation.

Results: SCI rats demonstrated pronounced, persistent motor deficits through day 14. Neurotropic autoantibody levels progressively increased, with the most pronounced rises in anti-opioid receptor IgG (2.4-fold, $p<0.05$), anti-NF200 IgG (by 50%, $p<0.05$), and anti-dopamine receptor IgG (by 45%, $p<0.01$). Spearman's analysis showed the strongest positive correlations with injury severity for anti-

*Corresponding Author: Kamol Ibrohimov, E-mail: ikamol030@gmail.com

GABA receptor IgG (r=0.962; p<0.001) and anti-β-endorphin IgG (r=0.851; p<0.001). Significant positive correlations were also found for anti-dopamine receptor IgG (r=0.685; p<0.001) and anti-S100B IgG (r=0.623; p<0.001).
Conclusion: Experimental SCI induces persistent motor deficits and progressive, marker-specific alterations in neurotropic autoantibody profiles involving structural proteins and neurotransmitter systems. These significant correlations indicate the potential value of the ELI-Neuro-Test panel as a multimarker approach for assessing neuroimmune alterations and experimental SCI severity.

Keywords
Spinal cord injury; neurotropic autoantibodies; ELI-Neuro-Test; motor function; neuroimmune response; biomarkers.

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Introduction

Traumatic spinal cord injury (SCI) remains one of the most challenging neurological conditions due to its complex pathophysiology, persistent functional deficits, and limited potential for neurological recovery. Despite substantial advances in acute care, surgical management, and rehabilitation, SCI frequently results in long-term motor, sensory, and autonomic dysfunction and imposes a considerable medical and socioeconomic burden. The biological consequences of SCI extend far beyond the initial mechanical damage, involving a complex sequence of molecular, cellular, inflammatory, and immune responses that may persist throughout the post-traumatic period [1,3,4].
The pathophysiology of SCI is generally characterized by primary and secondary injury mechanisms. Primary injury results from the immediate mechanical disruption of spinal cord tissue, whereas secondary injury involves a cascade of pathological processes, including vascular dysfunction, excitotoxicity, oxidative stress, mitochondrial impairment, blood–spinal cord barrier disruption, neuronal and glial cell death, and neuroinflammation [3,4]. These secondary processes may progressively expand the initial lesion and contribute substantially to neurological dysfunction and impaired locomotor recovery. At the same time, endogenous mechanisms of neural plasticity, axonal remodeling, and reorganization of spared neural circuits may promote varying degrees of functional recovery after SCI [7].
Neuroinflammation is recognized as a central component of the secondary injury response. Following SCI, resident microglia become activated, while circulating immune cells infiltrate the injured spinal cord and contribute to a dynamic inflammatory microenvironment [6,10]. Depending on the phase and severity of injury, these responses may exert both detrimental and reparative effects. Persistent activation of inflammatory pathways can promote neuronal and glial damage, demyelination, formation of inhibitory scar tissue, and chronic neurological dysfunction [5,10]. Moreover, prolonged interactions between the central nervous system and the systemic immune system may lead to immune dysregulation, including systemic immune suppression and autoimmune responses against neural antigens [5].
The development of reliable biomarkers reflecting the severity and progression of SCI has therefore become an important area of experimental and clinical research. Biomarkers detected in blood or cerebrospinal fluid may provide additional information about neural tissue damage, inflammatory activity, and the potential for neurological recovery [2,8]. Several molecular indicators associated with neuronal injury, astroglial activation, axonal degeneration, and inflammatory responses have been investigated as potential diagnostic and prognostic tools. However, considerable heterogeneity in injury mechanisms, sampling time points, analytical techniques, and outcome measures continues to limit the clinical and experimental interpretation of individual biomarkers [9].
Of particular interest is the post-traumatic immune response directed against nervous system antigens. Disruption of neural tissue integrity and alterations in the blood–spinal cord barrier may facilitate the exposure of normally sequestered neural antigens to the immune system. This process may contribute to the production of autoantibodies against neural proteins, receptors, and other components of nervous tissue. Persistent neuroinflammation and immune dysregulation may further modify these autoimmune responses and potentially influence secondary tissue damage and neurological recovery [5,6]. Nevertheless, the temporal patterns of neurotropic autoantibody responses following SCI and their relationship with changes in motor function remain insufficiently characterized.

Experimental models provide an important opportunity to investigate the dynamic relationship between molecular and immunological alterations and functional outcomes following SCI. In particular, repeated assessment of locomotor activity during the post-traumatic period enables the characterization of functional impairment and recovery, whereas simultaneous analysis of neural tissue-related biomarkers may provide insight into the biological mechanisms underlying these changes [3,4]. Integrating functional and immunological assessments may therefore contribute to a more comprehensive evaluation of injury severity and post-traumatic recovery.

Although considerable progress has been made in understanding the mechanisms of secondary injury, neuroinflammation, neural repair, and biomarker dynamics after SCI, the association between locomotor alterations and changes in neurotropic autoantibody profiles remains incompletely understood [2,7–9]. The evaluation of autoantibodies against multiple nervous system antigens may offer additional information about post-traumatic neuroimmune responses and their temporal evolution. In this context, the ELI-Neuro-Test represents an immunological approach for assessing changes in autoantibody profiles directed against nervous system-associated antigens.

Scientific Novelty: The scientific novelty of this study lies in the comprehensive and concurrent evaluation of motor coordination deficits, neuromuscular functional impairment, and tissue-specific alterations within central neuronal structures alongside systemic stress factors during SCI progression. For the first time, this study characterizes the temporal dynamics of neurotropic autoantibodies reflecting both adaptive and disadaptive central nervous system processes across acute and chronic post-traumatic phases, establishing their pathogenic correlation with specific locomotor activity parameters. Therefore, the present experimental study aimed to evaluate the dynamics of locomotor activity and neurotropic autoantibody profiles during the post-traumatic period in rats with spinal cord injury and to characterize the temporal changes in nervous system-related autoantibodies using the ELI-Neuro-Test.

MATERIALS AND METHODS

Experimental Animals and Group Allocation

The study was conducted in strict accordance with the requirements of the Bioethics Committee of the Ministry of Health of the Republic of Uzbekistan and Tashkent State Medical University. A total of 98 adult male outbred white laboratory rats with an initial body weight of 200–220 g were included in the experiments. The animals were housed under standard vivarium conditions at a constant temperature of 22 ± 2°C and a natural light–dark cycle, with free access to water and standard laboratory chow.

According to the type and severity of injury, the animals were allocated to the following groups:

1. Intact control group (n = 6): healthy animals that were not subjected to any mechanical injury.
2. Vertebral injury without spinal cord injury group (n = 20): injury was induced at the L3–L4 vertebral level by dropping a 250-g weight from a height of 20 cm, resulting in musculoskeletal injury without spinal cord damage.
3. Vertebral injury with spinal cord injury group (n = 20): severe mechanical contusion injury of the spinal cord was induced at the L3–L4 vertebral level by dropping a 250-g weight from a height of 40 cm.

The remaining animals were sequentially allocated to subgroups for morphological and biochemical analyses at different experimental time points.

Experimental Model of Spinal Cord Injury

Acute closed blunt mechanical injury of the vertebral column and spinal cord was induced using a specially designed vertical weight-drop device. The animals were immobilized under ether anesthesia, and the lumbosacral region corresponding to the L3–L4 vertebral level was identified. A cone-tipped metal cylinder weighing 250 g was directed through a vertical tube and allowed to fall freely onto the targeted region.

The severity of injury was controlled by varying the height from which the weight was dropped (20 or 40 cm). This experimental approach was designed to reproduce the principal features of contusion injuries that may occur in humans following road traffic accidents or falls from height [11, 12].

Assessment of Voluntary and Coordinated Motor Activity (Neuromotor Tests)

Post-injury neurological deficits and the dynamics of motor function recovery were assessed on days 3, 7, and 14 after injury using the following behavioral tests.

Voluntary locomotor activity. The voluntary motor activity of the animals was assessed using a Ugo Basile running wheel apparatus (Italy). The number and intensity of wheel rotations performed by each animal during a predefined observation period were recorded.

Motor coordination assessment. Static and dynamic balance and motor coordination were evaluated using a Rotarod+ apparatus (Russia). Motor performance was assessed by recording the time (in seconds) that each animal remained on the rotating rod. The apparatus was operated in an accelerating mode with a progressively increasing rotation speed. Post-injury neurological impairment and alterations in gait-related motor performance were quantitatively evaluated [11, 12].

Assessment of Neurotropic Autoantibody Dynamics Using the ELI-Neuro-Test

To assess the extent of neural tissue damage and the intensity of neuroimmune responses, serum levels of IgG autoantibodies directed against neurospecific antigens were determined using an enzyme-linked immunosorbent assay (ELISA). A commercially available ELI-Neuro-Test system (Russia) was used for the analysis.

The assay included the assessment of IgG autoantibodies against neurospecific proteins and neurotransmitter-related targets, including S100 protein, a marker associated with astroglial alterations; NF200, a neurofilament protein associated with axonal damage; myelin-related protein, associated with demyelinating processes; and glutamate, GABA, serotonin, dopamine, and opioid receptors, reflecting alterations in neurotransmitter systems.

Relative serum autoantibody levels were determined spectrophotometrically and expressed as percentages or arbitrary units relative to the mean values obtained in intact animals. The resulting immunological profile was used to assess neuroimmune alterations associated with neural tissue damage and post-traumatic processes [13].

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 26.0. The normality of data distribution was assessed using the Kolmogorov–Smirnov test. Depending on the distribution of the data, between-group differences were evaluated using parametric and non-parametric statistical methods, including Student's *t*-test, the Kruskal–Wallis test, and the Mann–Whitney *U* test with Bonferroni correction. Differences were considered statistically significant at $p < 0.05$.

Results

Dynamics of Voluntary Locomotor Activity After Experimental Spinal Injury

Dynamic assessment of voluntary locomotor activity after experimental spinal injury revealed significant differences between the groups in the severity of motor dysfunction and the rate of functional recovery. According to the study design, voluntary locomotor activity was assessed on days 3, 7, and 14 after injury using the Ugo Basile running wheel. The overall experimental design, allocation of animals into study groups, and time points of functional assessment are presented in Figure 1.

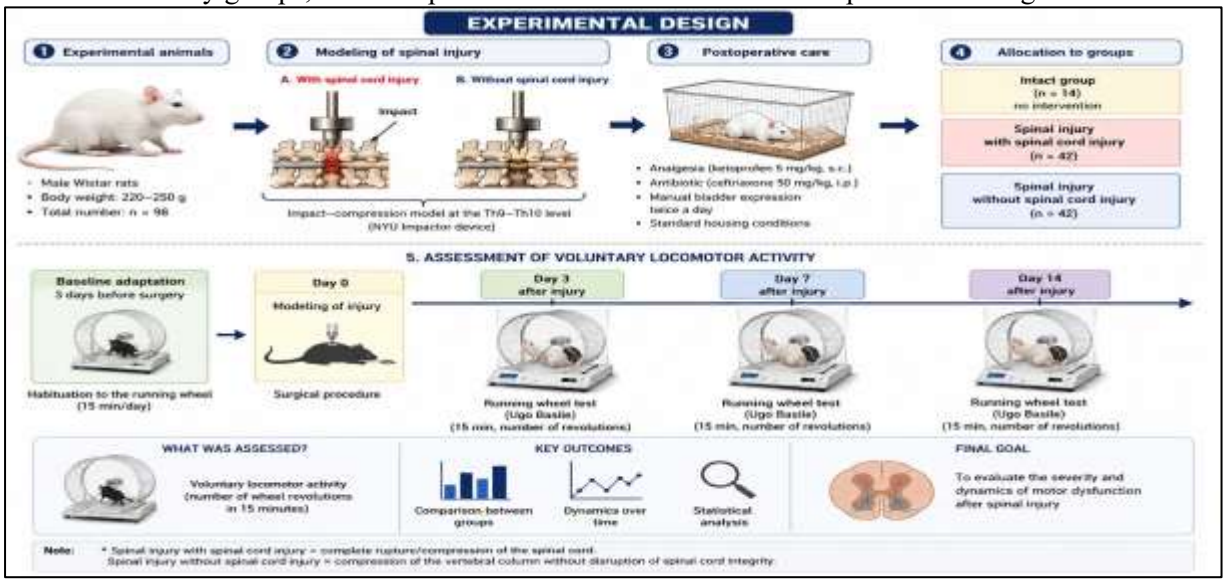

Figure 1. Experimental Study Design and Schematic Assessment of Voluntary Locomotor Activity Following Spinal Injury

A time-dependent pattern of changes in locomotor activity was observed throughout the follow-up period. The results obtained on days 3, 7, and 14 after injury demonstrated that motor recovery did not follow a linear trajectory but progressed at different rates depending on injury severity and the extent of spinal cord damage.

Persistent motor impairment was particularly evident in the group with spinal cord injury. In this group, locomotor activity remained lower than that of the intact control group at all observation time points, with the greatest functional deficit recorded during the early post-traumatic period. Although a partial recovery trend was observed at later time points, locomotor performance had not returned to baseline or intact control levels by day 14.

In contrast, animals with vertebral injury without spinal cord damage exhibited a more rapid recovery of locomotor activity. These findings indicate that direct spinal cord damage is a major pathogenic factor determining the severity and persistence of post-traumatic motor dysfunction (Table 1).

Table 1. Dynamics of Voluntary Locomotor Activity in the Experimental Groups (Number of Revolutions on the Ugo Basile Running Wheel, Mean ± SEM, n = 6–7)

Study group	Day 3	Day 7	Day 14
Spinal cord injury	10.6 ± 1.4*	18.6 ± 1.7*^	20.3 ± 2.6*^
Vertebral injury without spinal cord damage	14.8 ± 1.3*	28.3 ± 1.5	30.1 ± 2.1
Intact control	34.4 ± 1.9	34.4 ± 1.9	34.4 ± 1.9

Note: *p < 0.05 versus the intact control group; ^p < 0.05 between the spinal cord injury group and the vertebral injury without spinal cord damage group.

Comparative analysis of the data presented in Table 1 demonstrated a gradual recovery of voluntary locomotor activity in both experimental groups throughout the post-traumatic period. However, the rate of recovery differed markedly depending on the presence or absence of spinal cord damage. By day 14, locomotor activity in animals with vertebral injury without spinal cord damage approached the intact control level, whereas a substantial functional deficit persisted in animals with spinal cord injury. The statistically significant between-group differences observed on days 7 and 14 ($p < 0.05$) further indicate that direct spinal cord damage is an important determinant limiting post-traumatic motor recovery.

Dynamics of Hindlimb Muscle Strength Following Experimental Spinal Injury

To provide a comprehensive assessment of motor function, the dynamics of hindlimb grip strength and motor coordination were evaluated throughout the post-traumatic period. Both functional parameters showed marked impairment following spinal cord injury, although the magnitude and pattern of recovery differed between the experimental groups. Animals with vertebral injury without spinal cord damage demonstrated progressive recovery of hindlimb muscle strength and Rota-Rod performance from day 3 to day 14, whereas animals with spinal cord injury exhibited persistent functional deficits and substantially slower recovery. These between-group differences became particularly evident at the later observation time points, indicating that direct spinal cord damage markedly limits the restoration of muscle strength and motor coordination following experimental vertebral injury (Figure 2).

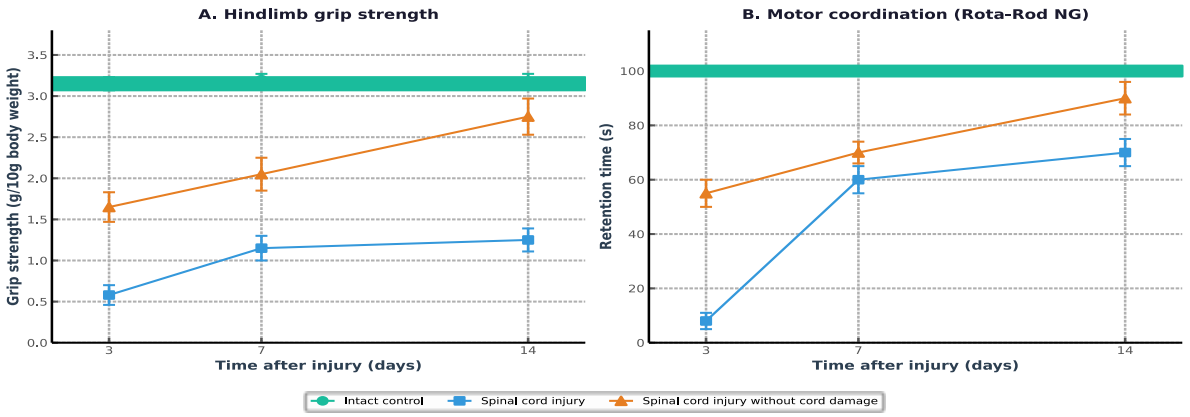

Figure 2. Dynamics of motor function after experimental vertebral injury: (A) hindlimb grip strength; (B) time spent on the rotating rod in the Rota-Rod NG test. Data are presented as M ± m (n = 6–7).

Dynamic assessment of hindlimb muscle strength revealed marked differences in the rate of functional recovery between the experimental groups. In animals with spinal cord injury, grip strength was 0.6 ± 0.2 g/kg on day 3 and increased to 1.2 ± 0.1 g/kg on day 7; however, only a minimal further increase to 1.3 ± 0.2 g/kg was observed by day 14, indicating a pronounced slowing of subsequent functional recovery. In contrast, animals with vertebral injury without spinal cord damage showed a progressive increase in muscle strength from 1.7 ± 0.3 g/kg on day 3 to 2.1 ± 0.3 g/kg on day 7 and 2.8 ± 0.3 g/kg on day 14.

Assessment of motor coordination using the Rota-Rod NG test further confirmed the differences in functional recovery between the groups. Animals with spinal cord injury exhibited a marked reduction in retention time on the rotating rod on day 3, followed by partial recovery at subsequent observation time points. Nevertheless, by day 14, motor coordination remained impaired compared with animals with vertebral injury without spinal cord damage. In contrast, the group without spinal cord damage demonstrated a progressive improvement in Rota-Rod performance throughout the entire observation period (Figure 2).

Combined analysis of grip strength and Rota-Rod performance demonstrated an initial trend toward functional recovery in both experimental groups; however, direct spinal cord damage markedly limited the subsequent rate and extent of motor recovery. In particular, the near-plateau in grip strength between days 7 and 14, together with persistent impairment of motor coordination, indicates sustained neurological dysfunction in animals with spinal cord injury.

Changes in the Neurotropic Autoantibody Profile

The post-traumatic dynamics of 12 neurotropic autoantibodies reflecting the functional status of the nervous system were evaluated in rats with spinal cord injury. The analysis included IgG autoantibodies against NF200, glial fibrillary acidic protein (GFAP), S100B, myelin protein, voltage-gated calcium channels, N-cholinergic receptors, glutamate receptors, GABA receptors, dopamine receptors, serotonin receptors, opioid receptors, and β-endorphin. Neurotropic autoantibody levels were determined using a multiparametric screening enzyme-linked immunosorbent assay. Changes in serum IgG autoantibody levels against neurospecific proteins and components of neurotransmitter systems in animals with spinal cord injury on days 3, 7, and 14 are presented in Table 2.

Table 2. Dynamics of ELI-Neuro-Test parameters in animals with spinal cord injury during the post-traumatic period

IgG autoantibodies	Day 3	Day 7	Day 14	p-value
Anti-NF200 IgG	0.405 (0.383–0.653)	0.496 (0.457–0.592)	0.607 (0.564–0.659)	<0.05
Anti-GFAP IgG	0.515 (0.505–0.550)	0.554 (0.531–0.650)	0.649 (0.609–0.763)	<0.05
Anti-S100B IgG	0.822 (0.794–1.096)	0.873 (0.841–1.096)	0.934 (0.884–1.303)	<0.01
Anti-myelin basic protein IgG	0.337 (0.296–0.365)	0.362 (0.341–0.409)	0.453 (0.418–0.477)	<0.01
Anti-voltage-gated calcium channel IgG	0.372 (0.322–0.394)	0.396 (0.357–0.429)	0.452 (0.419–0.496)	<0.01
Anti-N-cholinergic receptor IgG	0.490 (0.469–0.637)	0.534 (0.517–0.595)	0.576 (0.540–0.624)	<0.01
Anti-glutamate receptor IgG	0.408 (0.384–0.544)	0.459 (0.424–0.474)	0.489 (0.445–0.519)	<0.01
Anti-GABA receptor IgG	0.421 (0.384–0.621)	0.466 (0.432–0.473)	0.492 (0.438–0.559)	<0.01
Anti-dopamine receptor IgG	0.403 (0.393–0.632)	0.495 (0.432–0.597)	0.586 (0.553–0.650)	<0.01
Anti-serotonin receptor IgG	0.480 (0.415–0.582)	0.549 (0.528–0.613)	0.644 (0.602–0.703)	<0.01
Anti-opioid receptor IgG*	4.81 ± 0.36	10.90 ± 0.36	11.32 ± 0.15	<0.05

Note: Data are presented as median (interquartile range [IQR]) for non-normally distributed variables and as mean ± standard error of the mean (SEM) for normally distributed variables (*Anti-opioid receptor IgG). The p-values indicate the statistical significance of changes across the post-traumatic observation period (days 3, 7, and 14). Repeated-measures comparisons were performed using an appropriate parametric or non-parametric test according to the distribution of the data. A p-value <0.05 was considered statistically significant.

Dynamic analysis of the ELI-Neuro-Test parameters demonstrated a progressive increase in all investigated neurotropic autoantibody levels during the 14-day observation period following spinal cord injury. The most pronounced change was observed in anti-opioid receptor IgG levels, which increased 2.4-fold by day 14 compared with the initial measurement ($p < 0.05$). Anti-NF200 IgG, associated with axonal damage, increased by approximately 50% between days 3 and 14, whereas anti-GFAP IgG, reflecting astroglial alterations, increased by 26% ($p < 0.05$). In addition, anti-myelin protein IgG levels increased by approximately 34% ($p < 0.01$).

Among autoantibodies targeting neurotransmitter systems, anti-dopamine and anti-serotonin receptor IgG levels increased by approximately 45% and 34%, respectively. Statistically significant increases were also observed in autoantibodies against N-cholinergic, glutamate, and GABA receptors ($p < 0.01$), although the magnitude of these changes was less pronounced than that observed for the dopaminergic and serotonergic systems. Despite the relatively moderate increase in anti-S100B IgG, its elevated level persisted throughout the observation period.

Overall, the ELI-Neuro-Test results demonstrated a unidirectional but heterogeneous pattern of changes in the autoantibody profile following spinal cord injury. The most pronounced dynamics were observed for IgG autoantibodies against opioid receptors, NF200, and dopamine receptors, indicating that the post-traumatic neuroimmune response occurred concurrently with axonal damage and alterations in neurotransmitter systems.

Dynamic analysis of the ELI-Neuro-Test parameters revealed changes of varying magnitude in the levels of the investigated neurotropic autoantibodies during the post-traumatic period. To visually assess the time-dependent patterns of these changes, the dynamics of 12 neurotropic autoantibodies on days 3, 7, and 14 were visualized using a heatmap (Figure 2).

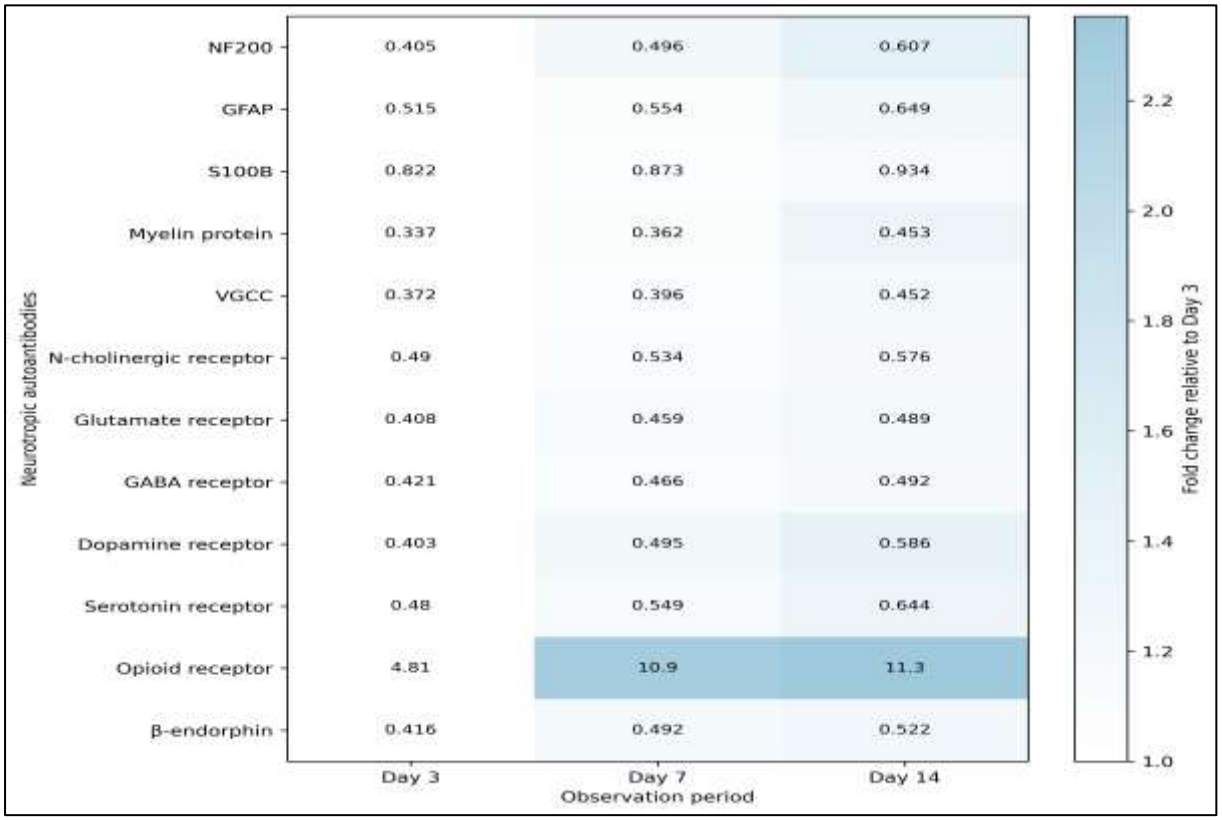

Figure 2. Heatmap visualization of temporal changes in neurotropic autoantibody levels following experimental spinal cord injury.

Time-dependent changes in neurotropic autoantibody levels following experimental spinal cord injury. The heatmap illustrates the dynamics of 12 neurotropic autoantibodies on days 3, 7, and 14 after injury. Values within the cells represent the autoantibody levels measured at the corresponding time points, whereas color intensity indicates the fold change relative to the day 3 value for each marker.

Elevated IgG autoantibody titers may be associated with the activity and severity of the post-traumatic inflammatory process. Spearman's correlation analysis revealed varying degrees of association between the levels of individual neurotropic autoantibodies and the severity of the pathological process in animals with spinal cord injury. In particular, anti-NF200 IgG levels showed a moderate positive correlation with axonal alterations ($r = 0.528$; $p < 0.001$). A weak positive correlation was observed between anti-opioid receptor IgG levels and the duration of the pathological process ($r = 0.293$; $p < 0.05$). The correlations between the neurotropic autoantibody profile and the severity of the post-traumatic process are presented in Table 3.

Table 3. Correlation between neurotropic autoantibody levels and the severity of the post-traumatic process

IgG autoantibodies	Spearman's r	p-value
Anti-NF200 IgG	0.528	<0.001
Anti-GFAP IgG	0.388	<0.001
Anti-S100B IgG	0.623	<0.001
Anti-myelin protein IgG	0.512	<0.001
Anti-voltage-gated calcium channel IgG	0.475	<0.001
Anti-N-cholinergic receptor IgG	0.396	<0.001
Anti-glutamate receptor IgG	0.481	<0.001
Anti-GABA receptor IgG	0.962	<0.001
Anti-dopamine receptor IgG	0.685	<0.001
Anti-serotonin receptor IgG	0.441	<0.001
Anti-opioid receptor IgG	0.293	<0.05
Anti-β-endorphin IgG	0.851	<0.001

Note: *r* — Spearman's rank correlation coefficient.

Spearman's correlation analysis revealed varying degrees of positive association between neurotropic autoantibody levels and the severity of the post-traumatic process. The strongest correlations were observed for anti-GABA receptor IgG ($r = 0.962$; $p < 0.001$) and anti-β-endorphin IgG ($r = 0.851$; $p < 0.001$). Relatively strong positive correlations were also found for anti-dopamine receptor IgG ($r = 0.685$; $p < 0.001$) and anti-S100B IgG ($r = 0.623$; $p < 0.001$). Anti-NF200 and anti-myelin protein IgG showed correlation coefficients of $r = 0.528$ and $r = 0.512$, respectively ($p < 0.001$). The weakest correlation was observed for anti-opioid receptor IgG ($r = 0.293$; $p < 0.05$). These findings indicate that the strength of the association between changes in neurotropic autoantibody profiles and the severity of the post-traumatic process varies considerably among individual markers.

Correlation analysis revealed distinct relationships between functional, electrophysiological, oxidative stress, and inflammatory and neurospecific parameters following experimental spinal cord injury. Functional recovery indices, including grip strength and Rota-Rod performance, were positively associated with electrophysiological measures of neuromuscular function and antioxidant defense markers, whereas inverse correlations were observed with oxidative stress, inflammatory, and neurospecific injury markers. Strong interrelationships were also identified among inflammatory mediators and markers of neuronal and glial damage, indicating coordinated progression of secondary neuroinflammatory and neurodegenerative processes following spinal cord injury (Figure 4).

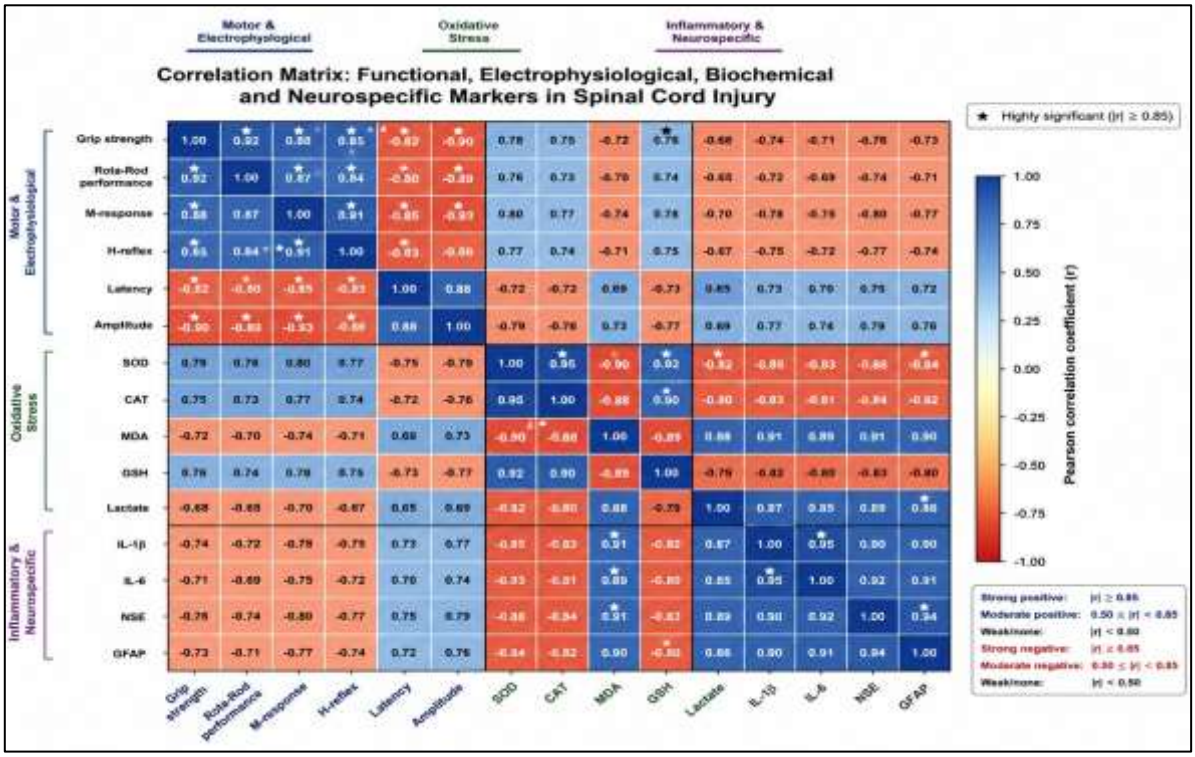

Figure 4. Correlation matrix of functional, electrophysiological, oxidative stress, inflammatory, and neurospecific markers following experimental spinal cord injury. Colors indicate the strength and direction of Pearson's correlation coefficients (r).

Correlation analysis revealed a strong association between motor recovery and electrophysiological function following spinal cord injury. Grip strength was strongly correlated with Rota-Rod performance (r=0.92), M-response (r=0.88), H-reflex (r=0.85), and response amplitude (r=0.90), whereas an inverse relationship was observed with latency (r=-0.82). Similarly, Rota-Rod performance correlated positively with M-response (r=0.87), H-reflex (r=0.84), and amplitude (r=0.89), confirming a close relationship between behavioral recovery and restoration of neuromuscular function.

Motor recovery was also associated with the balance between oxidative damage and antioxidant defense. Grip strength correlated positively with SOD (r=0.78), CAT (r=0.75), and GSH (r=0.76), but negatively with MDA (r=-0.72). A comparable pattern was observed for Rota-Rod performance, which correlated positively with SOD (r=0.76), CAT (r=0.73), and GSH (r=0.74), and negatively with MDA (r=-0.70). Within the oxidative stress profile, SOD and CAT showed a particularly strong positive correlation (r=0.95), whereas MDA was inversely correlated with SOD (r=-0.90), CAT (r=-0.88), and GSH (r=-0.89).

Inflammatory and neurospecific markers showed the opposite relationship with functional recovery. Grip strength was negatively correlated with IL-1β (r=-0.74), IL-6 (r=-0.71), NSE (r=-0.76), and GFAP (r=-0.73). Strong positive intercorrelations were observed between IL-1β and IL-6 (r=0.95), NSE and GFAP (r=0.94), and IL-6 and NSE (r=0.92). MDA was also strongly associated with IL-1β (r=0.91), NSE (r=0.91), and GFAP (r=0.90), linking increased lipid peroxidation to neuroinflammation and neuronal and glial injury.

Collectively, these findings demonstrate that impaired motor recovery after spinal cord injury is associated with delayed electrophysiological conduction, reduced antioxidant defense, enhanced lipid peroxidation, and increased inflammatory and neurospecific injury markers. The strongest correlations observed between SOD and CAT (r=0.95), IL-1β and IL-6 (r=0.95), and NSE and GFAP (r=0.94) indicate closely interconnected oxidative, inflammatory, and neurodegenerative responses during the post-traumatic period.

Discussion

The findings of this study demonstrate that functional impairment following experimental spinal cord injury is not limited to the consequences of primary mechanical damage but is accompanied by complex neuroimmune alterations that evolve over time. Animals with spinal cord injury exhibited marked

reductions in locomotor activity, hindlimb grip strength, and motor coordination, followed by incomplete functional recovery during the observation period. In particular, the failure of motor function to return to the levels observed in intact animals by day 14 indicates persistent functional impairment associated with secondary injury mechanisms and ongoing post-traumatic pathological processes.

In addition to functional impairment, the progressive changes observed in the neurotropic autoantibody profile indicate the potential involvement of immune mechanisms in the pathophysiology of spinal cord injury. Previous experimental studies have demonstrated that spinal cord injury induces prolonged B-cell activation and autoantibody production. Ankeny et al. showed that systemic autoimmune responses following spinal cord injury are associated with chronic B-cell activation, while subsequent studies demonstrated that pathogenic antibodies produced by these cells may impair functional recovery [14,15]. The progressive increase in neurotropic autoantibody levels from day 3 to day 14 observed in the present study is consistent with these findings.

However, increases in autoantibody levels should not be interpreted exclusively as evidence of a pathogenic autoimmune response. Arevalo-Martin et al. demonstrated that some autoantibodies detected during the subacute phase of spinal cord injury may represent naturally occurring antibodies [16]. This observation is important for interpreting our findings, as the dynamics of neurotropic autoantibodies may reflect both neural tissue injury and complex, marker-specific post-traumatic immune responses.

The time-dependent increases in IgG autoantibodies against NF200, GFAP, S100B, and myelin protein observed in the present study suggest ongoing post-traumatic alterations involving axonal, astroglial, and myelin-associated structures. These findings are consistent with previous studies demonstrating the diagnostic and prognostic relevance of nervous system-specific biomarkers following spinal cord injury. Pouw et al. identified alterations in structural biomarkers during the first 24 h after traumatic spinal cord injury, whereas Capirossi et al. demonstrated associations between early biomarker profiles and subsequent functional outcomes [18,19]. Our findings extend these observations by demonstrating that alterations in autoantibodies directed against neurospecific targets persist throughout the 14-day observation period.

The progressive increase in anti-NF200 IgG and its positive correlation with the severity of the post-traumatic process ($r = 0.528$; $p < 0.001$) may reflect ongoing axonal alterations. Similarly, the relatively strong correlation observed for anti-S100B IgG ($r = 0.623$; $p < 0.001$), together with changes in the anti-GFAP immune response, suggests the involvement of astroglial alterations during the post-traumatic period. These findings indicate that structural neural tissue damage and immune responses may represent interconnected rather than independent components of the post-traumatic pathological process.

One of the most notable findings of the present study was the very strong positive correlation between the severity of the post-traumatic process and IgG autoantibodies against GABA receptors ($r = 0.962$; $p < 0.001$) and β-endorphin ($r = 0.851$; $p < 0.001$). Significant positive correlations were also observed for anti-dopamine receptor IgG ($r = 0.685$; $p < 0.001$) and anti-S100B IgG ($r = 0.623$; $p < 0.001$). These findings suggest that post-traumatic neuroimmune alterations are not restricted to immune responses against structural neural proteins but may also involve neurotransmitter and neuromodulatory systems. This broader pattern may contribute to the complex mechanisms underlying post-traumatic motor dysfunction and functional recovery.

Recent evidence further supports the persistence of antineuronal immune responses following spinal cord injury. Schwab et al. demonstrated that local antibody synthesis and complement deposition within spinal cord lesions are associated with the development of de novo antineuronal autoantibodies [20]. The progressive increase in autoantibodies against 12 neurotropic targets and the significant correlations observed between several of these markers and the severity of the post-traumatic process are consistent with this concept. Collectively, these findings suggest that the immune response following spinal cord injury may represent an active component of post-traumatic pathophysiology rather than merely a secondary consequence of tissue damage.

Overall, our findings demonstrate a complex relationship among incomplete motor recovery, progressive alterations in neurotropic autoantibody levels, and the severity of the post-traumatic process. Previous studies have established the importance of B-cell activation, autoantibody production, and neuroimmune responses following spinal cord injury [14–16,20]. The present study extends these observations by simultaneously characterizing the dynamics of autoantibodies against NF200, GFAP, S100B, GABA receptors, and β-endorphin together with functional outcomes and the severity of the post-traumatic process. These findings support further investigation of neurotropic autoantibody

profiles as potential components of a multimarker approach for assessing the progression and severity of experimental spinal cord injury.

Conclusion

Experimental spinal cord injury resulted in persistent impairment of locomotor activity, hindlimb strength, and motor coordination, accompanied by progressive increases in IgG autoantibodies against neural structural proteins and components of neurotransmitter systems during the 14-day observation period. These findings indicate that post-traumatic functional impairment is accompanied by sustained and marker-specific neuroimmune alterations.

The strongest positive correlations with the severity of the post-traumatic process were observed for anti-GABA receptor IgG ($r = 0.962$) and anti-β-endorphin IgG ($r = 0.851$; both $p < 0.001$). These findings suggest that ELI-Neuro-Test parameters may have potential value as components of a multimarker approach for evaluating neuroimmune alterations and the severity of experimental spinal cord injury. Further studies with larger sample sizes and independent validation are required to determine their diagnostic and prognostic utility.

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Ethical Approval
The experimental study was reviewed and approved by the Local Ethics Committee of the Tashkent Medical Academy under the Ministry of Health of the Republic of Uzbekistan (Extract from Minutes No. 6 dated June 25, 2025; Approval No. 14).

Conflict of Interest
The authors declare no conflict of interest.

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