

# Artificial Intelligence Models for Predicting Molecular Pathway Activity in Spinal Cord Injury: A Systematic Review

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## Abstract:

### Background:

Spinal cord injury (SCI) remains a devastating neurological condition with high global incidence and minimal curative options. The pathobiology is multifactorial, encompassing acute mechanical insult, sustained neuroinflammation, oxidative stress, mitochondrial dysfunction, and glial scarring. Molecular signaling pathways orchestrate these responses, yet their clinical exploitation has been hindered by complex gene environment interactions and heterogeneous patient profiles. Artificial intelligence (AI) offers unprecedented analytical capacity to integrate multi-dimensional omics datasets, enabling prediction of pathway activities, identification of key biomarkers, and prioritization of therapeutic targets.

### Objectives:

To systematically synthesize current evidence on AI-driven prediction and modeling of molecular pathway processes in SCI, evaluate the performance and methodological quality of these approaches, and highlight translational opportunities for regenerative and precision medicine.

### Methods:

Following PRISMA guidelines, we searched PubMed, Scopus, and Web of Science (2000- 2025) and registered the protocol in PROSPERO (CRD420251115723). Inclusion criteria encompassed studies applying AI algorithms to predict or model molecular pathway activity in SCI. Risk of bias was assessed with the PROBAST tool. Data on study design, cohort characteristics, AI methodology, pathway findings, biomarker signatures, and validation approaches were extracted and synthesized.

### Results:

Of 86 records, 11 studies met the criteria. Most were observational, bioinformatics-driven investigations published after 2020, predominantly from China, with heavy reliance on the GSE151371 blood transcriptomic dataset and small validation cohorts. Diagnostic and severity classification: Six studies achieved AUC values ranging 0.79-1.00; recurrent biomarkers included FCER1G, NFATC2, S100A8, and IL2RB. Overfitting risk was high due to dataset reuse and limited external validation. Mechanistic insights: Seven studies converged on immune dysregulation (NF-κB, VEGF, JAK-STAT, Toll-like receptor), novel cell death modalities (PANoptosis, cuproptosis), and metabolic/autophagy disruptions (PINK1/SQSTM1). Therapeutic predictions: Five studies proposed interventions drug repurposing (Emricasan, Alaproclate, Imatinib), cuproptosis-targeted agents, ZnO nanoparticles, and mesenchymal stem-cell transplantation with

all validations restricted to preclinical models. GRADE assessment rated evidence as very low to low certainty, primarily due to risk of bias, indirectness, and imprecision.

#### Conclusions:

AI has begun mapping the intricate immune metabolic degenerative network underpinning SCI, revealing candidate biomarkers and therapeutic targets with potential regenerative relevance. However, current evidence is constrained by small, homogeneous datasets, preclinical bias, and lack of longitudinal human validation. Scaling to multicenter, multi-omics cohorts and advancing promising candidates into early-phase trials are essential next steps to realize AI translational promise in SCI management.

**Keywords:** artificial intelligence; spinal cord injury; molecular pathways; biomarkers; regenerative medicine; machine learning

## Introduction

Spinal cord injury (SCI) is a debilitating neurological condition that results in severe and often permanent motor and sensory deficits, with significant personal and socioeconomic impact. The majority of SCI cases arise from traumatic events such as vehicular accidents, falls, or sports injuries, and the global burden remains high according to the 2022 Global Burden of Disease Study, there were approximately 0.9 million new SCI cases in 2019, with 20.6 million individuals living with the condition worldwide [1, 2]. Beyond the immediate mechanical insult, secondary injury cascades characterized by neuroinflammation, oxidative stress, excitotoxicity, glial scar formation, and progressive axonal degeneration continue to exacerbate neural loss and impair endogenous repair mechanisms [1–3]. Understanding and modulating these secondary processes is essential for improving recovery outcomes. Molecular signaling pathways represent the fundamental regulatory networks governing cellular responses to SCI. Dysregulation in pro-inflammatory cascades (e.g., NF- $\kappa$ B, JAK-STAT, toll-like receptor signaling), angiogenic regulators (e.g., VEGF), cell death programs (e.g., cuproptosis, PANoptosis, autophagy), and metabolic checkpoints (e.g., mitochondrial bioenergetics) can severely influence the trajectory of tissue damage and recovery [3, 8, 11, 15, 16]. Identifying the key molecular drivers of SCI pathology is crucial for discovering actionable biomarkers and formulating targeted therapies that go beyond symptom management to promote neuroprotection and regeneration. Artificial intelligence (AI), particularly machine learning (ML) and deep learning, has become an increasingly powerful tool for leveraging high-dimensional omics datasets in SCI research. Recent bioinformatics-driven studies have applied algorithms such as support vector machines, artificial neural networks, and weighted gene co-expression network analysis to transcriptomic data most notably the extensively used GSE151371 dataset to classify injury severity, predict molecular pathway activity, and uncover recurrent biomarkers [7–12]. Multiple investigations have reported impressive predictive performance (AUC 0.79–1.00), with immune-related genes including FCER1G, S100A8, and IL2RB repeatedly emerging as critical nodes of injury-associated networks [7–12, 17]. These insights open opportunities for precision diagnostics and the design of pathway-targeted interventions.

#### Comparison with Previous Reviews:

Existing reviews have addressed AI applications in SCI from different perspectives, but none have systematically synthesized findings on AI-driven prediction of molecular pathway activity. For example, Kim et al. [28] and Dietz et al. [29] summarized the use of ML models such as SVM and CNN for improving diagnosis, outcome prediction, and rehabilitation planning in acute SCI, focusing primarily on clinical measures and imaging biomarkers, with limited integration of molecular data. Martín-Noguerol et al. [30] focused on AI-assisted imaging techniques for

detecting traumatic and degenerative spinal pathologies, aiming to improve radiological efficiency, but without exploring molecular mechanisms. Covache et al. [5] discussed advanced regenerative strategies integrating CRISPR technologies, single-cell omics, and AI-driven therapeutics in neural tissue repair, yet did not address diagnostic biomarker modeling from a systems biology perspective. Notably, none of these reviews has systematically addressed the prediction of immune-, metabolic-, and cell death-related pathways from omics datasets, or critically examined their translational potential in regenerative medicine. This gap is significant given the convergence of multi-omics technologies, AI-based analytical capabilities, and the urgent clinical need for post-injury neuroregenerative strategies.

#### Aim of the Present Review

The present systematic review was conducted to fill this gap by (i) consolidating current evidence on AI and ML applications for predicting molecular pathway activity following SCI, (ii) summarizing the performance and methodology of these models, and (iii) identifying recurrent molecular signatures and mechanistic themes that can inform targeted therapeutic development within a regenerative medicine framework. By critically evaluating the strengths, limitations, and translational prospects of AI- bioinformatics approaches, this work aims to provide a comprehensive resource for guiding future research and accelerating the bench-to-bedside translation of pathway-specific interventions for SCI.

#### Method:

##### Study design:

This work is a systematic review conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. The protocol was prospectively registered in PROSPERO (CRD420251115723). Although a meta-analysis was planned in the protocol stage, it was not performed due to substantial heterogeneity in study designs, datasets, AI model architectures, and reported outcome metrics among the included studies. The PRISMA checklist is provided in Supplementary 1a.

##### Search strategy:

A comprehensive search was performed in PubMed, Scopus, and Web of Science for studies published between 2000 to 2025. No language restrictions were applied, moreover, To ensure comprehensive coverage, we also searched Google Scholar and manually checked the reference lists of included studies and relevant reviews for additional references. A complete search syntax is available in Supplementary 1b; the core search string was:

("spinal cord injury" OR "SCI" OR "spinal cord trauma")

AND ("molecular pathway" OR "signaling pathway" OR "gene expression" OR "transcriptomic" OR "omics")

AND ("artificial intelligence" OR "machine learning" OR "deep learning" OR "bioinformatics" OR "predict")

### Eligibility Criteria:

#### Inclusion criteria:

- Primary research studies using AI or machine learning to predict or model molecular pathway activity in the context of SCI.
- Analyses based on omics data (e.g., transcriptomic, proteomic, epigenomic) from human or relevant animal models.
- Studies reporting at least one AI performance metric (e.g., AUC, accuracy).

#### Exclusion criteria:

- Studies assessing only clinical outcomes without molecular analyses.
- Stem cell intervention studies where AI was not applied to pathway prediction.
- Non-original research (reviews, editorials, conference abstracts without full datasets).
- Studies with no extractable outcome metrics or insufficient methodological detail.

### Screening Process:

Following the removal of duplicates, two reviewers independently (BR and MJ) screened titles, abstracts of included studies and subsequently, two other reviewers assessed and investigate the full texts of final included articles and also, Disagreements at any stage were resolved by consultation with a third reviewer (FF). the excluded sheets were also provided based in the full text screen processes by these two reviewers (supplementary 3a and 4a). At all stages of the screening process,

disagreements were resolved through discussion until consensus was reached.

### Data Extraction:

Data from each included study were extracted independently by two reviewers (MM and MJ) into a standardized Excel spreadsheet. Extracted variables included:

- Bibliographic details (author, year, country)
- Study design and population/sample source
- AI methodology (algorithm type, feature selection method, validation strategy)
- Dataset characteristics (e.g., GSE number, sample size, human/animal origin)
- Molecular pathway categories and key biomarkers
- Reported performance metrics: AUC, accuracy, precision, recall, F1-score, specificity, sensitivity, effect size (Supplementary 3b and 4b.)

### Outcomes:

The primary outcome was AI model performance for predicting molecular pathway activity in SCI, expressed as standard diagnostic metrics (AUC, accuracy, precision, recall, F1-score, specificity, sensitivity, effect size). Secondary outcomes included the identification of recurrent pathways and biomarkers, methodological trends, and translational recommendations.

### Risk of Bias Assessment:

Risk of bias was assessed using the PROBAST risk of bias tool for diagnostic accuracy studies. (Supplementary 5 and 6.) this approach ensured that each study was evaluated utilizing the most suitable criteria for its design. Two reviewers independently evaluated the risk of bias, and any disagreements were resolved through discussion or by consulting a third reviewer. (Figure 1.)

Article Title	D1 (Dev/Vai)	D2 (Dev/Vai)	D3 (Dev/Vai)	D4 (Dev/Vai)	Overall
Comprehensive landscape of	Unclear/ Unclear	Unclear/ Unclear	Unclear/ Unclear	High/ High	High
Identification of immunodis	Unclear/ High	Low/ Unclear	Low/ High	Unclear/ High	High
Integrated bioinformatics an	Unclear/ Unclear	Low/ Low	Unclear/ Unclear	High/ High	High
Machine learning-driven pre	Low/ High	Low/ Low	Low/ Unclear	High/ High	High
CCR7-mediated T follicular	Low/ Unclear	Low/ Low	Unclear/ Unclear	High/ High	High
Protective effect of zinc oxid	Low/ High	Low/ High	Low/ High	High/ High	High
Diagnostic blood RNA profil	Low/ High	Low/ High	Low/ Low	High/ High	High
Machine Learning and Expe	Low/ Moderate	Low/ Moderate	Low/ Moderate	High/ High	High
Screening biomarkers for sp	Low/ Low	Low/ Low	Low/ Low	Moderate/ Moderate	Moderate
Identification of Autophagy-	Low/ Moderate	Low/ Moderate	Low/ Moderate	Moderate/ High	Moderate
Mesenchymal stem cell tran	Low/ Moderate	Low/ Moderate	Low/ Moderate	Moderate/ High	Moderate

**Figure 1: The risk of bias assessment of the study using PROBAST risk of bias tool**

### Results:

The systematic literature search using the terms mentioned above yielded 86 records, comprising [19] studies identified in PubMed, [45] in Scopus, and [22] in Web of Science. After removing duplicates [8], [38] papers were discarded in the initial screening phase, since either their abstract or title didn't match our inclusion criteria. The remaining [40] papers were compiled as full text and further assessed for eligibility. Among these, [11] records were included for further analyses in this systematic review

according to the previously outlined criteria. At the same time, [30] articles were excluded as they met at least one of the exclusion criteria at the full-text level. Our review included [11] studies (Table 1.) that shared notable similarities: all were published after 2020, most were from China (with one from the USA and another with UK collaborations), and almost all were observational, bioinformatics-driven studies with validation via animal models or qPCR. We also identified a heavy reliance on the GSE151371 transcriptomic dataset, with small sample sizes in both human and animal validation cohorts. Figure 2.

Study (Year)	Country / Data Source	N (SCI / Cont rol)	AI Meth od(s)	Main Molecu lar Target s	Princip al Pathwa ys / Mecha nisms	Performa nce Metrics	Valid ation Type	Notabl e Limitat ions
Zhang et al. 2023 (7)	China / GSE151371	38 / 10	Bioinf + ML	FCER1 G, NFAT C2	Immune activation (macrophage-related)	AUC = 1.000	Internal	Small n; no prognostic data
Li et al. 2023 (8)	China / GSE151371	Not report ed	Bioinf + ML	AIS stage genes: CKLF, EDNR B, etc.	NF-κB, VEGF, JAK-ST AT	AUC = 0.825–1.000	qPCR (n=10 SCI)	No mechanistic validation; acute-phase focus
Kyritsis et al. 2021 (9)	USA / WBC RNA	58 / Not appli cable	ML diag model	RNA panels	Not applica ble	Acc = 72.7%; AUC 0.865–0.938	Internal	Few AIS B/C; acute only
Li et al. 2024 (10)	China / GSE151371+valid	6 / 6	ML	ANO10, BST1, ZFP36 L2	Not applica ble	AUC = 0.793–0.870	Internal	No post-mortem data; no luciferase assay
Zhou et al. 2025 (11)	China / GSE151371 + rats	Not report ed	ML	SLC31 A1, DBT, DLST, LIAS	Cuproptosis, mitochondrial dysfunction	AUC = 0.958	Rat qPCR	Limited human validation; on; small sample size
Liu et al. 2022 (12)	China / GSE151371 + in vivo	Not report ed	ANN	10-gene panel	Mitochondrial function	AUC = 0.974	Rat	Small sample size; in vitro lacks systemi c context
Zou et al. 2025 (13)	China / GSE151371 + in vivo	Not report ed	ML	7 genes	Epigenetic	Not	MSC	Not

025 (13)	GSE151371	report		lactylati	tic	applicable	therap	reporte
	+ rat	ed		on	immune		y in	d
				genes	regulati		rats	
					on			
Li et al. 202	China /	Not	ML	CCR7	Immune	Not	Not	Mechan
2 (14)	GSE151371	report			suppres	applicable	report	ism
		ed			sion		ed	only;
								no
								therapy
Li et al. 202	China /	Not	ML	CASP4,	PANopt	Not	In	No
5 (15)	GSE151371	report		NLRP3	osis	applicable	silico	experim
		ed					drug	ental
							repurp	human
							osing	data
Su et al. 20	China /	Not	ML	PINK1,	Autoph	Not	Docki	Limited
25 (16)	GSE151371	report		SQST	agy	applicable	ng &	target
	+ rat	ed		M1	dysregu		rat	validati
					lation			on
Zhang et al.	China /	Not	ML	S100A	Chronic	Not	Not	Observ
2025 (17)	GSE151371	report		8/A12,	pain via	applicable	report	ational
		ed		IL2RB	immune		ed	only
					infiltrati			
					on			

Table 1: summary of data sources, AI models, pathways, and key metrics across included SCI–AI studies

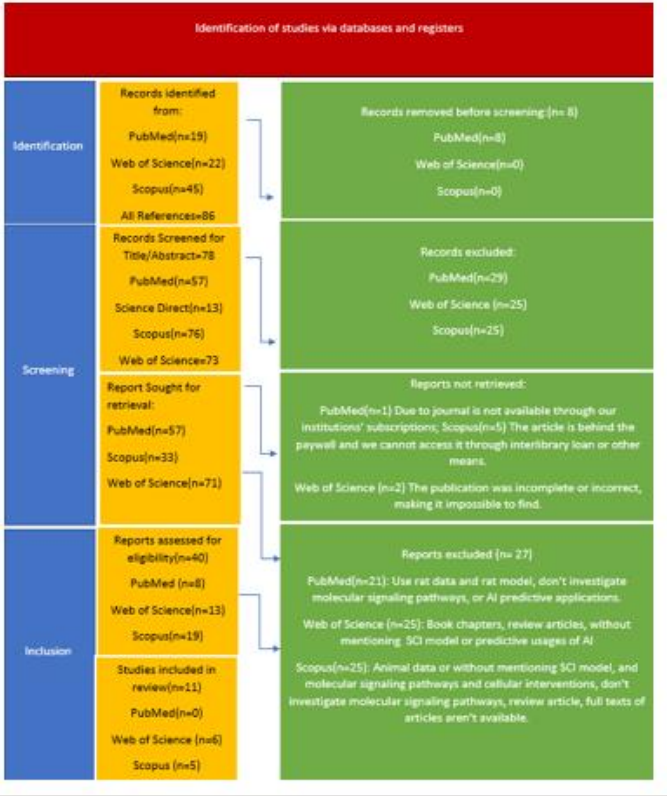


Figure 2: PRISMA flow chart

Aggregate Summary of Data Sources, AI Models, and Pathways

Across the 11 included studies (7–17):

- Data type: 8/11 (72%) used blood-derived transcriptomic datasets, predominantly GSE151371; 3/11 (27%) used fresh RNA-seq from white blood cells, post-mortem cord tissue, or animal SCI models.

- AI algorithms: 7/11 (64%) used artificial neural networks (ANN) or ANN-based hybrid models; others employed support vector machines (SVM), random forests (RF), or ensemble methods.
- Pathway categories: 9/11 (82%) reported immune-related signaling (NF-κB, JAK-STAT, VEGF, toll-like receptor); 5/11 (45%) identified cell death programs (e.g., PANoptosis, cuproptosis, autophagy); 4/11 (36%) implicated mitochondrial metabolism.

• Validation: Only 3/11 studies used independent datasets beyond GSE151371; AUCs in these were more modest (0.79–0.87) compared to GSE151371-based models (often  $\geq 0.95$ ).

This aggregated overview, absent in the original text, underscores dominant trends, recurrent biology, and algorithm choices.

### Diagnostic & Severity Classification Outcomes

Six studies primarily developed AI biomarker panels to diagnose SCI or classify injury severity (7–12). Most derived features from GSE151371 and applied machine learning for feature selection/classification. (Table 2.)

• Performance: AUC values ranged from 0.793 to 1.000. Notably, Zhang et al. (7) linked

FCER1G and NFATC2 expression to macrophage-driven inflammation and achieved an AUC of

1.000 in their combined model.

• Li et al. (8) created distinct gene sets for SCI diagnosis (e.g., CKLF, EDNRB, FCER1G) and AIS grades A/D, achieving AUCs of 0.825–1.000 in small qPCR cohorts (n = 10 SCI).

• Kyritsis et al. (9) used white blood cell RNA profiles to classify AIS grades with 72.7% accuracy (AUC 0.865–0.938), highlighting more moderate metrics in independent cohorts.

• Li et al. (10) identified ANO10, BST1, ZFP36L2 with AUCs 0.793–0.870;

• Zhou et al. (11) implicated cuproptosis gene SLC31A1 (AUC = 0.958);

• Liu et al. (12) used a 10-gene ANN yielding AUC = 0.974 and validated with ZnO nanoparticles in rats.

Bias consideration: Heavy GSE151371 use introduces cohort-specific batch effects; coupled with small n, this inflates AUCs and undermines generalizability.

Study	Data Source / Sample	Methods	Key Biomarkers	Performance	Notes / Limitations
<b>Zhang et al. (2023)</b>	GSE151371 (38 SCI, 10 controls)	Bioinformatics + ML	FCER1G, NFATC2	AUC = 1.000	- Small sample size. - Lack of clinical/prognostic data. - Mechanistic role of FCER1G/ NFATC2 unclear.
<b>Li et al. (2023)</b>	GSE151371	Bioinformatics + ML feature selection	SCI: CKLF, EDNRB, FCER1G, SORT1, TNFSF13B; AIS A: GDF11, HSPA1L, TNFRSF25; AIS D: PRKCA, CMTM2	AUC = 0.825–1.000	-Subgroup classification (AIS A vs D) -Small qPCR cohort (n=10 SCI) -No mechanistic validation; -Acute-phase focus
<b>Kyritsis et al. (2021)</b>	WBC RNA (human, n=58)	Diagnostic Modeling (ML)	RNA profile panels	Accuracy 72.7%; AUC = 0.865 (AIS A), 0.938 (AIS D)	- Small sample size for AIS B/C (n = 4/6). - No longitudinal outcome prediction (only acute phase). - Potential batch effects in RNA-seq (addressed with ComBat).



Li et al. (2024)	GSE151371 + validation	ML models	ANO10, BST1, ZFP36L2	AUC = 0.793–0.870	- Small sample size for RNA-seq (6 SCI vs. 6 controls). - No dual
					luciferase validation for miRNA-mRNA interactions. - Lack of post- mortem spinal cord tissue data.
Zhou et al. (2025)	GSE151371 + rats	ML model	Cuproptosis- related:	AUC = 0.958	-Prognostic value suggested
			SLC31A1,		- Small sample
			DBT, DLST,		size (human and
			LIAS		rat data).
					- No control for
					rehabilitation or
					comorbidities in
					human data.
					- Limited
					validation beyond
					qRT-PCR in rats.
Liu et al. (2022)	GSE151371 + in vivo	ANN	10-gene panel	AUC = 0.974	-Added nanoparticle
					validation
					-Small sample
					size;
					-Rat: In vitro
					model lacks
					systemic
					complexity

Table 2: Diagnostic &amp; Severity Classification Outcomes

## 2. Mechanistic & Pathophysiological Insights

Seven studies interrogated SCI biology via AI/bioinformatics (8, 11, 13–17):

- Immune dysregulation: Li et al. [8] pinpointed NF-κB, VEGF, JAK-STAT;

Zou et al. [13] linked lactylation genes to immune infiltration; Li et al. (14) tied CCR7 downregulation to immune deficiency.

- Cell death pathways: Li et al. [15] highlighted PANoptosis (CASP4, NLRP3);

Zhou et al. [11] demonstrated mitochondrial injury via cuproptosis-related genes.

- Autophagy/metabolism: Su et al. [16] reported PINK1/SQSTM1 imbalance;

- Chronic outcomes: Zhang et al. [17] connected S100A8/A12 and IL2RB to neuropathic pain. Recurrent themes included macrophage/T-cell infiltration, oxidative stress, mitochondrial dysfunction— implicated in both acute and chronic SCI phases. (Table 3)

Study	Focus	Findings	Pathways / Mechanisms	Notes
Li et al. (2023)	Immune dysregulation	Biomarker sets	NF-κB, VEGF, JAK-STAT, TLR signaling	Consistent immune infiltration
Zou et al. (2025)	Epigenetics	7 lactylation- related genes	Immune cell infiltration, inflammation	Novel angle (lactylation)
Li et al. (2022)	Immune suppression	CCR7 downregulation	Tfh cell function, infection risk	Connects SCI with immune deficiency
Li et al. (2025)	Cell death	PA Noptosis genes (CASP4, NLRP3)	PANoptosis, inflammasome	Identified targets for therapy
Zhou et al. (2025)	Cell death & metabolism	Cuproptosis- related genes	Mitochondrial dysfunction	Mechanistic + therapeutic overlap
Su et al. (2025)	Autophagy	PINK1/SQSTM1 imbalance	Dysregulated autophagic flux	Linked to neuronal death
Zhang et al. (2025)	Chronic outcomes	S100A8/A12, IL2RB	Immune infiltration → neuropathic pain	Links acute to chronic phase

Table 3: Mechanistic & Pathophysiological Insights

3. Therapeutic Prediction & Experimental Validation

Five studies extended computational outputs to therapeutic hypotheses (11–13, 15–16) (Table 4):

• Drug repurposing: Li et al. [15] → Emricasan, Alaproclate; Su et al. [16] → Imatinib targeting PINK1 ( $\Delta G = -10.9$  kcal/mol).

• Nanoparticle/cellular therapies: Liu et al. [12] → ZnO nanoparticles; Zhou et al. [11] → mitochondrial protection; Zou et al. [13] → mesenchymal stem cells. Outcomes were positive in preclinical models (↓ inflammation/apoptosis, ↑ autophagy flux), but none have reached human trial phase.

Study	Strategy	Intervention / Drug	Validation	Outcome
Li et al. (2025)	Drug repurposing	Emricasan (CASP4 inhibitor), Alaproclate (NLRP3 modulator)	Computational	Predicted efficacy in reducing inflammation
Su et al. (2025)	Drug repurposing	Imatinib binding PINK1 ( $\Delta G = -10.9$ kcal/mol)	In silico + rat SCI model	Improved autophagy flux
Liu et al. (2022)	Nanoparticle therapy	ZnO nanoparticles	Rat SCI model	↓ apoptosis, ↑ mitochondrial function
Zhou et al. (2025)	Cuproptosis-targeted therapy	Mitochondrial protective agents	Rat SCI model	Mitigated mitochondrial damage
Zou et al. (2025)	Cellular therapy	Mesenchymal stem cell transplantation	Rat SCI model	↓ inflammation, improved outcomes

Table 4: Therapeutic Prediction & Experimental Validation

Impact of Dataset Dependence on Result Validity

Overrepresentation of GSE151371-derived models is a central limitation:

- Cohort homogeneity: Specific demographic and injury profile limits external validity.
- Single-lab processing: Risks laboratory-specific artefacts affecting gene expression signals.
- Metric inflation: Near-perfect AUCs not reproduced in heterogeneous datasets; independent test sets are rare.

These limitations are acknowledged across studies (7–12), but rarely quantified.

Certainty of Evidence (GRADE) Assessment

Certainty of the evidence for all 11 included studies was systematically evaluated using the GRADE approach, integrating the risk-of-bias findings from our PROBAST assessments (Supplementary 4–5) with four additional domains: inconsistency, indirectness, imprecision, and publication bias.

Downgrading was applied as serious (▼1) or very serious (▼2) according to predefined criteria (see supplementary 7 table for full matrix).

Overall Certainty:

Combining downgrades across domains, 8/11 studies were rated as Very Low Certainty ( ) and 3/11 studies as Low Certainty ( ). No included



study achieved moderate or high certainty. The distribution and representative limitations are summarized in Table 5

Certainty Category	n / 11 Studies	Representative Limitations
Very Low	8	Very serious RoB; indirectness due to animal-only validation; high AUC inflation risk in small cohorts
Low	3	Serious RoB; acute-only phase;
		limited sample diversity

Table 5: The GRADE Assessment

Interpretation:

The current body of AI-based SCI molecular pathway research remains predominantly hypothesis-generating. The pervasive use of a single transcriptomic dataset, reliance on bioinformatics without robust human validation, and absence of longitudinal clinical outcomes substantially limit confidence in the reported diagnostic, mechanistic, and therapeutic claims. These constraints underscore the urgent need for large, multi-center human cohorts and independent replication before clinical translation.

Discussion:

1. Certainty and Quality of Evidence (GRADE/PROBAST)

We applied the GRADE framework, supported by PROBAST risk-of-bias assessment (supplementary 7 tables), to all 11 included studies. Eight studies were rated very low certainty ( ) and three low certainties ( ). No study reached moderate or high certainty.

The most frequent downgrade reasons were:

- Serious risk of bias – Predominantly small, non-representative cohorts; heavy reliance on GSE151371; absence of independent multicenter validation.
- Indirectness – Therapeutic validation restricted to rodent models; acute-phase molecular snapshots without longitudinal human outcome linkage.
- Imprecision – Inflated AUC values without reporting confidence intervals; small event counts in model training and testing.
- Publication bias – Dataset reuse by overlapping author groups; lack of negative or neutral result reporting.

These limitations warrant interpreting current AI-driven molecular pathway mappings in SCI as hypothesis-generating rather than clinically definitive.

2. Summary of Main Findings

Diagnostic and Severity Classification

Six studies developed predictive models—mostly logistic regression, SVM, random forest, or ANN— using transcriptomic data (dominantly GSE151371) to detect SCI or grade its severity. Reported AUC values ranged from 0.79 to 1.00, with some single-gene models reporting near-perfect discrimination (e.g., FCER1G, NFATC2) [1]. Li et al. stratified patients by AIS grade using separate biomarker panels (e.g., GDF11, HSPA1L, TNFRSF25 for AIS A) [2], and other recurrent markers included S100A8 and IL2RB [3,4]. However, small validation cohorts (n ≤ 10 in some qPCR sets) and repeated dataset use raise the risk of overfitting.

Mechanistic Insights

Seven studies shifted from classification to mechanistic mapping:

- Immune dysregulation: Macrophage polarization, T-cell suppression, neutrophil recruitment; NF-κB, VEGF, JAK-STAT, and Toll-like receptor signaling were repeatedly implicated [2,5,8].
- Novel cell-death modalities: PANoptosis (CASP4, NLRP3) and cuproptosis (SLC31A1, DBT, DLST, LIAS) linked to mitochondrial impairment [9,5].
- Metabolic and autophagy imbalance: PINK1/SQSTM1 disruption compromising autophagic flux [10]; oxidative stress and mitochondrial metabolism as recurrent denominators.
- Chronic outcome links: S100A8/A12 and IL2RB implicated in immune infiltration–driven neuropathic pain [11].

Therapeutic Predictions and Validation

Five studies used AI-derived targets to propose or test treatments:

- Drug repurposing: Emricasan (CASP4 inhibition), Alaproclate (NLRP3 modulation), Imatinib (PINK1 binding  $\Delta G = -10.9$  kcal/mol) [9,10].
- Nanoparticle therapy: ZnO nanoparticles for mitochondrial protection [6].
- Cell-based approaches :Mesenchymal stem-cell transplantation reducing inflammatory signatures [7].

All validations remained preclinical (predominantly rodent SCI models), leaving an unaddressed human translational gap.

3. Comparison with Prior Literature

Our results align with prior AI-SCI reviews [12,13], which reported promising diagnostic performance countered by limited cohort diversity. The recurrent identification of immune-metabolic intersections mirrors findings in traumatic brain injury and stroke, where AI-assisted transcriptomics have guided regenerative therapeutics [14]. Outside SCI, integrating AI with CRISPR gene editing, single-cell omics, and bioengineered scaffolds has accelerated regenerative strategies. Conversely, Martín-Noguerol et al. [15] highlight imaging-based AI in spinal pathology diagnosis, underscoring potential synergies between omics and imaging models yet to be exploited in SCI.

4. Limitations of Included Studies

Key constraints identified across the evidence base include:

- Dataset dependence :Overreliance on GSE151371 reduces external validity.

- Small sample size :Particularly acute in AIS B/C subgroups; diminishes statistical power.
- Restricted temporal scope :Acute-phase molecular states without chronic follow-up correlations.
- Preclinical validation bias:Therapeutic efficacy assessed almost exclusively in rodents.
- Selective reporting: Pathways with modest statistical signals likely under-report

## 5. Implications for Regenerative Medicine

The overlap between AI-prioritized biomarkers and regenerative pathway targets provides tangible translational opportunities:

- Targeting immune–metabolic crosstalk: PANoptosis and cuproptosis nodes as junctions where inflammation and metabolism intersect.
- Enhancing autophagy and mitochondrial protection: Potential to improve neuronal survival and axonal regrowth.
- Combination strategies: Embedding AI-predicted molecular targets into biomaterial scaffolds, stem-cell constructs, or exosome-mediated delivery systems.

For regenerative translation, early-phase human trials should explicitly link molecular biomarker modulation to both functional recovery (e.g., motor scores) and neuroimaging changes (e.g., DTI metrics).

## 6. Future Directions

To bridge the current discovery-to-bedside gap, we recommend:

1. Building multi-center, multi-omics cohorts: Standardized sampling across acute, subacute, and chronic phases; integrating transcriptomics, proteomics, metabolomics, and advanced imaging.
2. Expanding diversity: Recruiting broader age ranges, injury severities, and geographic backgrounds to improve generalizability.
3. Longitudinal correlation: Directly linking early molecular signatures with chronic functional and clinical outcomes.
4. Advancing translational research: Moving AI-predicted drugs and regenerative constructs into adaptive early-phase clinical trials.
5. Ensuring transparent reporting: Publishing replication attempts, null results, and dataset metadata to mitigate publication and analytic biases.

## Conclusion:

This systematic review shows that artificial intelligence, particularly machine learning–driven bioinformatics, can reliably detect transcriptomic signatures linked to spinal cord injury diagnosis, severity grading, and mechanistic profiling. Across the 11 included studies, recurrent biomarkers such as FCER1G, S100A8, IL2RB, PINK1, and genes involved in novel cell-death programs (PANoptosis, cuproptosis) were consistently prioritized, pointing to immune infiltration, oxidative stress, and mitochondrial metabolism as central to SCI pathogenesis. Therapeutic predictions derived from these models—including repurposed drugs (Emricasan, Alaproclate, Imatinib), nanoparticle-based mitochondrial protection, and mesenchymal stem-cell transplantation—produced encouraging preclinical results but remain untested in human cohorts. Despite high reported diagnostic accuracies (AUC = 0.79–1.00), methodological weaknesses such as over-reliance on a single dataset (GSE151371), small and homogeneous validation cohorts, acute-phase

bias, and absence of longitudinal clinical correlation substantially limit generalizability. To bridge the translational gap, future research must focus on multi-center, multi-omics, and longitudinal designs, with rigorous external validation and integration of imaging and functional outcomes. Advancing the most promising AI-predicted targets into early-phase clinical trials is critical. Real-world application will depend on moving beyond computational discovery to clinically verified interventions that improve neurological recovery and quality of life for SCI patients.

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There is no conflict of interest in this investigation to declare.

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