Review

Application value of biofluid-based biomarkers for the diagnosis and treatment of spinal cord injury

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Abstract

Recent studies in patients with spinal cord injuries (SCIs) have confirmed the diagnostic potential of biofluid-based biomarkers, as a topic of increasing interest in relation to SCI diagnosis and treatment. This paper reviews the research progress and application prospects of recently identified SCI-related biomarkers. Many structural proteins, such as glial fibrillary acidic protein, S100-β, ubiquitin carboxy-terminal hydrolase-L1, neurofilament light, and tau protein were correlated with the diagnosis, American Spinal Injury Association Impairment Scale, and prognosis of SCI to different degrees. Inflammatory factors, including interleukin-6, interleukin-8, and tumor necrosis factor α, are also good biomarkers for the diagnosis of acute and chronic SCI, while non-coding RNAs (microRNAs and long non-coding RNAs) also show diagnostic potential for SCI. Trace elements (Mg, Se, Cu, Zn) have been shown to be related to motor recovery and can predict motor function after SCI, while humoral markers can reflect the pathophysiological changes after SCI. These factors have the advantages of low cost, convenient sampling, and ease of dynamic tracking, but are also associated with disadvantages, including diverse influencing factors and complex level changes. Although various proteins have been verified as potential biomarkers for SCI, more convincing evidence from large clinical and prospective studies is thus required to identify the most valuable diagnostic and prognostic biomarkers for SCI.

Key Words: biomarker; diagnosis; inflammatory cytokine; motor recovery; non-coding RNA; prognosis; spinal cord injury; structural protein; trace element

Introduction

Spinal cord injuries (SCIs) are severe complications of spinal injuries caused by e.g. car accidents and falls, which are accompanied by clinical manifestations including spinal cord concussion and spinal shock (McDonald and Sadowsky, 2002; Deng et al., 2020). SCIs cause devastating and long-term disabilities, and improving the prognostication of patients has thus become a long-standing goal in clinical research (Fakhoury, 2015; Eckert and Martin, 2017; Liu et al., 2017). The main clinically proven effective treatment strategies for SCI currently include early decompression, exercise rehabilitation, drug therapy, and cell transplantation (McDonald and Sadowsky, 2002; Guo, 2020; Huang et al., 2020; Sharif and Jazaib Ali, 2020). However, there is still a lack of timely prevention strategies for secondary SCI and of effective treatment measures to achieve satisfactory recovery (Hu et al., 2021).

At present, the presence of typical neurological symptoms combined with spinal cord imaging results are considered as effective and authoritative methods for diagnosing SCI (Mortazavi et al., 2015; Fan et al., 2018). However, the accurate judgement of injury severity and SCI prognosis based on imaging performance and physical examination are limited. Among the available imaging tools, magnetic resonance imaging (MRI) remains the most objective and authoritative method for diagnosing SCI, by providing high-resolution and contrast images of the injured spinal cord (Freund et al., 2019). However, early MRI is not always suitable or accessible in patients with unstable SCIs (Wang et al., 2018; Albayar et al., 2019; Seif et al., 2019). Regarding physical examination, although the International Standards for Neurological Classification of Spinal Cord Injury examination is generally considered as the gold standard and is widely applied for the assessment of SCI severity and prognosis (Hales et al., 2015; Chun et al., 2020), this relatively subjective evaluation has certain limitations associated with its time-consuming nature and the need for patient cooperation, which is not applicable to patients in a state of coma, sedation, or spinal shock (Thomas and Murphy, 2018).

The above limitations indicate the urgent need to find other auxiliary indicators to accurately evaluate the degree of injury and clinical prognosis of patients with SCI. Numerous recent studies have revealed that some structural proteins

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and inflammatory factors in cerebrospinal fluid (CSF) and serum can reflect the degree of SCI (Badhiwala et al., 2018). However, although many researchers have reviewed the changes of these biomarkers in SCI, few have focused on which biomarkers are the most beneficial for the diagnosis of SCI, the grading of American Spinal Injury Association (ASIA) Impairment Scale (AIS), and for assessing the prognosis of SCI, which are the most important clinical applications of biomarkers. In this review, we summarize and review recent progress in relation to the common biofluid-based biomarkers with applications in SCI.

Search Strategy and Selection Criteria

HDW and ZJW performed a literature search of PubMed, Web of Science, and Google Scholar using the following search terms: "spinal cord injury" "biomarkers" "GFAP" "S100β" "NSE" "UCH-L1" "NF" "tau" "MMPs" "IL-6" "IL-8" "TNFα" "IL-1β" "IGF-1" "TGF-β" "non-coding RNAs" "miRNAs" "lncRNAs". Articles focusing on the role of biomarkers in the diagnosis, AIS classification, and motor recovery of SCI were included. Overall, 75 studies published between 2002 and 2021 were included.

Structural Biomarkers for Spinal Cord Injury

During SCI, neuronal and glial cell damage results in the release of cellular components into the CSF and peripheral circulation through the ruptured blood-spinal cord barrier. The concentrations of these cellular components change over time, making them potentially useful indicators of the severity of SCI and recovery prognosis. Protein biomarkers can conventionally be divided into structural and inflammation-related biomarkers (Kwon et al., 2019). The different pathophysiological processes involved in producing these biomarkers include astroglial injury (S100 calcium-binding protein β, S100β; glial fibrillary acidic protein, GFAP), neuronal cell-body damage (neuron-specific enolase, NSE; ubiquitin C-terminal hydrolase-L1, UCH-L1), axonal damage (neurofilament proteins, NF; myelin basic protein, MBP), and dendritic damage (microtubule-associated protein) (Wang et al., 2018) (**Figure 1**). Based on the current research results, we divided protein biomarkers into (1) biomarkers related to the diagnosis of SCI, (2) biomarkers related to American Spinal Injury Association (ASIA) Impairment Scale (AIS) classification, and (3) biomarkers related to the prognosis of SCI. We have summarized and discussed the biomarkers in each category, with the aim of identifying two or three biomarkers or their combinations with the greatest clinical potential (**Table 1**).
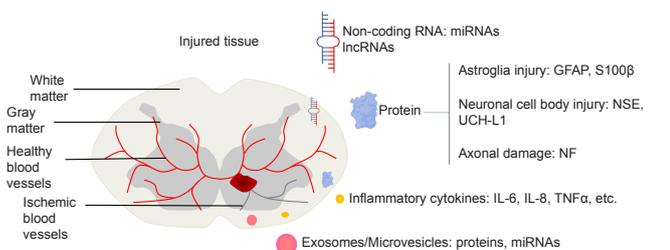

Figure 1 | Classification of spinal cord injury (SCI) biomarkers.
SCI biomarkers can be divided into several categories, including structural proteins, inflammatory cytokines, non-coding RNAs, and microvesicles. Protein SCI biomarkers can be divided into three categories based on the processes in which they are involved: astroglial injury (S100β, GFAP), neuronal cell body injury (NSE, MBP), and axonal damage (NF). GFAP: glial fibrillary acidic protein: IL: interleukin; lncRNA: long non-coding RNA; miRNA: microRNA; NF: neurofilament proteins; TNFα: tumor necrosis factor α.

Astroglial Injury Biomarkers for Spinal Cord Injury

GFAP exists in the astrocyte matrix and is a single-molecule intermediate filament protein with high specificity. It has been confirmed as a robust biomarker of traumatic brain injury (TBI) (Wang et al., 2018) and may also have great value for the assessment of SCI. Kwon et al. (2017) performed CSF GFAP testing and AIS grading in 15 patients with acute SCI at 24 hours and 6 months post-injury. They demonstrated that CSF GFAP concentrations were significantly different among patients diagnosed with AIS grades A, B, and C at 24 hours after SCI injury, and GFAP levels were also significantly different between patients with an improved AIS grade after 6 months of treatment compared with those with no improvement. In their study, GFAP had an 83% probability of predicting neurological improvement in patients with AIS grade A at 6 months after injury, indicating that GFAP might be a useful index for assessing the severity of SCI and for predicting future neurological functional recovery (Kwon et al., 2017). Guéz et al. (2003) showed that GFAP levels were higher in patients with complete motor loss compared with patients with incomplete motor loss. Ahadi et al. (2015) also reported that a higher degree of SCI was associated with higher serum concentrations of GFAP in a study of 35 patients. Obly et al. (2019) recently found that serum GFAP concentrations rose in the first 1–3 days in 31 dogs with simulated SCI, and then gradually decreased and became undetectable by 14 days. Importantly, the serum level of GFAP in the first 3 days predicted recovery with an accuracy of 76.7–86%. Regarding sequelae for patients with chronic SCI, Holmstrom et al. (2020) found that CSF GFAP and phosphorylated neurofilament-heavy (pNF-H) levels were significantly higher in 12 patients with spinal cord tethering compared with radiculopathy controls. Collectively, GFAP has thus been shown to be a strong prognostic biomarker for neurological amelioration after SCI.

S100β is a calcium-binding protein that is mainly present in astrocytes and Schwann cells, and participates in calcium homeostasis, cell proliferation, and differentiation (Donato et al., 2009). Similarly, S100β has been confirmed as a biomarker of TBI and also has an essential role in SCI diagnosis (Hulme et al., 2017). Kwon et al. (2010) detected CSF expression levels of S100β and other cytokines in 27 patients with SCI, and found that combining GFAP with IL-8 and S100β predicted the observed ASIA grade with 89% accuracy at 24 hours and better predicted segmental motor function improvement after 6 months of SCI injury compared with baseline ASIA grade. Similar conclusions were reached in a series of subsequent studies (Wolf et al., 2014). Kwon et al. (2017) verified that CSF S100β levels differed significantly among patients with different AIS grades of SCI at 24 hours post-injury, and S100β was also significantly correlated with improvements in sports scores and the prediction of ASI conversion over 6 months. In a recent study in 60 patients, serum S100β increased after SCI and reached a peak 4 days after injury, and then declined to a relatively low level 2 weeks later (Du et al., 2018). Du et al. (2018) also showed that SCI patients who showed improvements of at least one ASIA grade after 6 months had lower S100β levels than those whose ASIA grade changed by less than one grade throughout the course of the disease. However, despite the diagnostic sensitivity of S100β for SCI, S100β also exists in adipocytes and chondrocytes, and increased serum levels of S100β alone thus lack specificity for diagnosing SCI (Faridaalee and Keyghobadi Khajeh, 2019).

Neuronal Cell Body Injury Biomarkers for Spinal Cord Injury

The glycolytic enzyme NSE is mainly distributed in the cytoplasm of mature neurons and neuroendocrine cells, and is released into the extracellular environment following neuronal axon damage. In an early study of 34 patients with vertebral spine fractures, Wolf et al. (2014) found no difference in serum NSE levels between experimental and normal subjects or among SCI patients classified with paresthesia, incomplete

Table 1 | Summary of structural proteins and inflammatory cytokines in recent human SCI studies published during 2010–2021

Reference	Phase of SCI	Testing method	Biomarker	Groups and sample size — Control group	Study group	Type of sample	AIS grade	Time of sampling	Role in diagnosis of SCI	Role in AIS grading of SCI	Role in the prediction of SCI recovery	Conclusion
Kwon et al., 2010	Acute and subacute	Bio-Plex system (Bio-Rad, Hercules, CA, USA) using a 25-plex human cytokine kit and Meso Scale Discovery® platform; ELISA	IL-6, IL-8, MCP1, Tau, S100β, GFAP	27 patients with complete SCI (ASIA A) or incomplete SCI (ASIA B or C)		CSF	A–C	At 0–24, 24–48, 72–96 and > 96 h	Not described	CSF, IL-6, IL-8, MCP-1, tau, s100beta, and GFAP levels were elevated in a severity-dependent fashion. A biochemical model using s100beta, GFAP, and IL-8 can predict injury severity (ASIA A, B, or C)	Segmental motor recovery at 6 mon post- injury was better predicted by these CSF proteins than with the patients' baseline ASIA grade	CSF, IL-6, IL-8, MCP-1, tau, s100beta, and GFAP can stratify injury severity and predict neurological outcome
Hayakawa et al., 2012	Acute and subacute	ELISA	Pnf-H	14 patients with acute cervical SCI		Plasma	A–D	At 6, 12, 18, 24, 48, 72 and 96 h, and 6, 8,10, 14 and 21 d after injury	Not described	Plasma pnf-H level was elevated in accordance with the severity	Not described	Pnf-H is a potential biomarker to independently distinguish AIS A patients (complete SCI) from AIS C–E patients (incomplete SCI)
Pouw et al., 2014	Acute	ELISA	NSE, S100β, NF-H, GFAP	16 patients with traumatic SCI		CSF	A–D	Within 24 h after injury	Not described	CSF, NSE, S100β, NF-H concentration in 'motor complete' (AIS A, B) patients than in 'motor incomplete' (AIS C, D) patients	Not described	Structural CSF biomarkers NSE, S-100β and NFH were different between motor complete and motor incomplete SCI patients
Wolf et al., 2014	Acute	ELISA	NSE, S100β	29 patients with proximal femur fractures	34 patients with acute vertebral fractures	Serum	Not described	Within 24 h after injury	Serum S00β level was significantly increased in patients with vertebral spine fractures	Not described	Not described
Ahadi et al., 2015	Acute and subacute	ELISA	GFAP, pnf-H, NSE	9 patients with spinal fractures but without neurological symptoms	35 patients	Serum	A–D	At 24, 48 and 72 h after injury	Serum GFAP, pnf-H and NSE levels were significantly higher in SCI patients than in controls	Serum level of GFAP differed between motor-complete SCI (AIS A and B) and motor-incomplete SCI (AIS C and D)	The serum level of GFAP was appropriate for estimating the severity of SCI in the first 24 hours after injury.
Bank et al., 2015	Acute and subacute	Multiplex assays (Bio-Plex Pro Human Cytokine 21-Plex, Group II, #MFO-005KMII and 27-Plex, Group I #M500KCAF0Yb)	MIF and 47 other factors	18 uninjured participants	18 adults with acute traumatic SCI	Plasma	A–D	At 0–3, 4–7, 8–11 and 12–15 d	MIF, IL-6, IL-9, IL-16, IL-18, chemokines, growth-related oncogene α/chemokine (C-X-C motif) ligand1, macrophage inflammatory protein 1-β/chemokine (C-C motif) ligand 4, hepatocyte growth factor and stem cell growth factor-β increased significantly after injury.	Not described	Not described
Biglari et al., 2015	Acute, subacute and intermediate	The Quantikine Human Immunoassay (R&D Systems, Inc, Minneapolis, MN,USA)	IL-1β, TNF-α	23 patients (group 1: with AIS improvement; group 2: without AIS improvement)		Serum	A–D	At 0, 4, 9 and 12 h, 1 and 3 d and at 1, 2, 4, 8 and 12 wk	Not described	Not described	Patients with AIS improvement (group 1) had significantly lower TNF-α levels at 9 hours compared with patients without AIS improvement (group 2; P < 0.01)	Measuring serum levels of TNF-α and IL-1β over time can be useful in tracking the course of SCI.
Kuhle et al., 2015	Acute and subacute	Nflumea47:3 (an immunoassay developed in-house for nfl)	NF-L	67 healthy controls	Patients with central cord syndrome (n = 4), motor incomplete SCI (n = 10), motor complete SCI (n = 13)	Serum	A–D	At 0, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156 and 168 h after injury	Baseline nfl level was higher in patients with motor incomplete SCI (21 pg/mL) and in patients with motor complete SCI (70 pg/mL) than in healthy controls (5 pg/mL) and central cord syndrome (6 pg/mL).	Nfl level was correlated with ASIA motor score at baseline (r = –0.53) and after 24 h (r = –0.69).	Nfl level was correlated with 3-12-mon motor outcome (baseline nfl: r = –0.43, P = 0.026 and 24-h nfl: r = –0.72, P < 0.001).
Moghaddam et al., 2016	Acute, subacute and intermediate	ELISA	IGF-1	45 patients (G1: remission; G2: no remission)		Serum	A–D	At 0, 4, 9 and 12 h, 1, 3, 7 and 14 d and 1, 2 and 3 mon	Not described	Patients with AIS A grade had higher level of IGF-1 than patients with B-D grade at 7 d and 2 mon.	Patients with clinically documented neurological remission showed consistently higher IGF-1 level than patients without neurological remission.	Serum sustained increased IGF-1 indicted neurological remission.
Kwon et al., 2017	Acute	Bio-plex (Bio-Rad) multiple magnetic bead antibody detection assay on inflammatory cytokines; ELISA for tau, GFAP, and S100β detection	IL-6, IL-8, MCP-1, Tau, S100β, GFAP	50 acute SCI patients		CSF	A–C	At 24 h after injury	Not described	IL-6, tau, S100β, and GFAP levels were significantly different between patients with baseline AIS A, B, or C	IL-6, IL-8, MCP-1, tau, S100β, and GFAP levels were significantly different between those who improved an AIS grade over 6 mon and those who did not improve.	The analysis of CSF can provide valuable biological information about injury severity and recovery potential after acute SCI.
Moghaddam et al., 2017	Acute, subacute and intermediate	Luminex Performance Human High Sensitivity Cytokine Panel	MMP-2, MMP-8, MMP-9, MMP-10, MMP-12	Control (10 fracture patients without neurological impairment)	10 patients with traumatic SCI with neurological remission (group 1) and 10 patients with traumatic SCI without neurological remission (group 0)	Serum	Not described	At 0, 4, 9 and 12 h, 1 and 3 d and 1, 2, 4, 8 and 12 wk after injury	Not described	Not described	Not described	Favored predictive model including MMP-8 value 1 day and MMP-9 value 1 month after SCI enables a prediction of neurological remission in 97% of cases.
Dalkilic et al., 2018	Acute	Bio-plex (Bio-Rad) multiple magnetic bead antibody detection assay on inflammatory cytokines; ELISA for tau, GFAP, and S100β detection	IL-6, IL-8, MCP-1, Tau, GFAP, S100β	36 acute cervical SCI patients		CSF	A–C	At 24 h after injury	Not described	CSF biomarker level was found to correlate with baseline injury grade. Combination of MRI and CSF biomarkers provides a stronger model for classifying baseline AIS grade than CSF or MRI biomarkers alone.	Inflammatory CSF biomarkers best predicted AIS grade conversion, whereas structural biomarker levels best predicted motor score improvement.	The CSF biomarker discriminated better between different injury severities, and is a stronger predictor of neurological recovery in terms of AIS grade and motor score improvement than MRI
Du et al., 2018	Acute and subacute	ELISA	NSE, S100β	60 patients with acute SCI were divided into group A (1 or more than 1 ASIA grade improved at 6 months after the injury) and group B (ASIA grades changed < 1 at 6 months after injury)		Serum	Not described	At 0, 2, 4, 6, 8, 10, 12, 14 d after injury	Not described	Not described	Serum NSE and S100β levels predicted the prognosis of acute SCI patients with the sensitivity of 74.35% and 71.79%, the specificity of 71.43% and 66.67%, respectively.	Serum NSE and S100B protein levels can reflect the degree of SCI and could be potential biomarkers for the prognosis of acute SCI.
De Mello Rieder et al., 2019	Acute and subacute	ELISA	NSE, IL-6, NGF, GDNF	36 healthy subjects	52 acute SCI patients	Serum	Not described	Within 48 h and 7 d after injury	Serum NSE level increased while NGF decreased at 48 h and 7 d; serum IL-6 level increased only at 48 h.	Not described	Not described
Holmström et al., 2020	Intermediate and chronic	ELISA	GFAP, UCH-L1, Pnf-H	7 patients with benign lumbar intradural tumors and 7 patients with cervical radiculopathy	12 patients with spinal cord tethering	CSF and serum	A–D	1-24 m	CSF GFAP (P ≤ 0.01) and CSF pnf-H (P ≤ 0.05) levels were increased in patients compared with radiculopathy controls.	Not described	Not described
Ogurcov et al., 2021	Subacute	Bio-Plex Pro™ Human Cytokine 40-plex Assay #171AK99MR2 (Bio-Rad) on cytokines; ELISA for NSE and VEGF detection	Interferon-γ, CCL27, CCL26, CXCL5, CCL11, CXCL11, IL-10, TNF-α, MIF, NSE, VEGF	16 healthy subjects	28 patients with SCI	Serum	A–B	2 wk after injury	Serum interferon-γ (> 52 fold), CCL27 (> 13 fold), and CCL26 (> 8 fold) increased; serum NSE increased after SCI.	Serum CXCL5, CCL11, CXCL11, IL-10, TNF-α, and MIF were different between patients with AIS A and B.	Not described
Stukas et al., 2021	Acute and subacute	UCH-L1 Discovery Assay (102343) from Quanterix Corporation (Billerica, MA, USA)	UCH-L1	10 patients with spinal stenosis and lumbar disc herniations	32 acute SCI patients	CSF and serum	A–C	At 24, 48, 72, and 96 h after injury	CSF UCH-L1 elevated by 10- to 100-fold over laminectomy controls	CSF UCH-L1 increased in an injury severity-dependent manner.	100% sensitivity and 86% specificity in the prediction of motor recovery at 6 mon after injury

AIS: American Spinal Injury Association (ASIA) Impairment Scale; ASIA: American Spinal Injury Association; CSF: cerebrospinal fluid; Cu: copper; ELISA: enzyme-linked immunosorbent assay; GFAP: glial fibrillary acidic protein; IGF-1: insulin-like growth factor 1; IL: interleukin; MCP-1: monocyte chemotactic protein-1; Mg: magnesium; MIF: macrophage migration inhibition factor; MMP: matrix metalloproteinase; NF-L: neurofilament protein-light; NSE: neuron-specific enolase; pnf-H: phosphorylated neurofilament-heavy; S100β: S100 calcium-binding protein β; SCI: spinal cord injury; Se: selenium; TGF-β: transforming growth factor β; TNF-α: tumor necrosis factor α; UCH-L1: ubiquitin carboxy-terminal hydrolase L1; Zn: zinc.

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paraplegia, and complete paraplegia. However, Ahadi et al. (2015) subsequently arrived at different conclusions based on significantly higher average serum NSE levels in 35 patients with SCI, compared with the control group at 24 and 48 hours post-injury. Similarly, the results of a study by Pouw et al. (2014) based on 16 patients with acute traumatic SCI showed that CSF levels of NSE were significantly correlated with baseline neurological functions, such as complete motor loss (AIS grade A or B) or incomplete motor loss (AIS C or D). However, recent studies have produced conflicting results: a study of 52 patients by de Mello Rieder et al. (2019) found that serum NSE levels were significantly increased at 48 hours and 7 days after SCI, but found no significant difference between patients with different ASIA grades and no significant correlation between post-injury serum NSE and future neurologic melioration.

However, in a study of 60 patients with acute SCI by Du et al. (2018), serum NSE levels were significantly increased after injury and reached a peak on the 2nd day, and then declined gradually. Furthermore, patients with an improvement of at least one ASIA grade 6 months later had lower serum NSE levels than those who did not recover well (less than one grade improvement). In summary, NSE could serve as a diagnostic biomarker for SCI, and possibly also for severity assessment and prognostic evaluation.

UCH-L1 is a deubiquitinase involved in the addition or removal of ubiquitin in proteins that primarily exists in the cytoplasm of neuronal cells. Yang et al. (2018) showed that CSF and serum UCH-L1 levels peaked rapidly at 4 hours after injury then declined at 24 hours in a rat SCI model. In addition, a recent study of 32 patients with acute SCI revealed that CSF UCH-L1 levels increased dramatically after injury in a severity- and time-dependent way, with 100% sensitivity and 86% specificity for predicting ASIA conversion, despite no differences in serum UCH-L1 levels. Furthermore, a consistent increase in CSF UCH-L1 up to 96 hours and relatively higher CSF levels 24 hours after injury were correlated with a lack of AIS grade improvement and motor function amelioration, respectively (Stukas et al., 2021). An ultrasensitive immunosensor has recently been developed for the rapid detection of UCH-L1 in biofluids in patients with TBI or SCI (Khetani et al., 2019). Overall, CSF levels of UCH-L1 have great potential for the diagnosis of acute SCI, and might qualify as a sensitive biomarker for predicting future neurological recovery.

Axonal Damage Biomarkers for Spinal Cord Injury

NF, as a major component of the axon cytoskeleton, interacts with other cytoskeletal proteins and regulates axonal transportation and neuronal signaling (Yuan et al., 2017). NF exists exclusively in neurons but is released and increased in the extracellular environment following neuronal cell damage, and is thus a potential biomarker for SCI (Al-Chalabi and Miller, 2003). The basic structural components of NF include three polypeptide subunits: neurofilament-light (NF-L, 68K), neurofilament-medium (150K), and neurofilament-heavy (NF-H, 200K), among which phosphorylated NF-H (pNF-H) is most easily detectable in serum and CSF due to its relative resistance to proteases (Shaw et al., 2005). Hayakawa et al. (2012) recruited 14 patients with acute cervical SCI and found that serum pNF-H was detectably increased from 12 hours to 21 days after injury in patients with complete SCI, and there were significant differences in serum pNF-H levels between patients with AIS grade A (completely paralyzed) and AIS grade C (incompletely paralyzed). A later study involving 35 patients also found that serum pNF-H levels were significantly higher in patients with SCI at 24 and 48 hours after injury compared with controls (Ahadi et al., 2015). In addition,

serum NF-L has been identified as a potential biomarker in various neurological diseases (Gaetani et al., 2019). In an early clinical study of SCI biomarkers, Guez et al. (2003) examined CSF samples from six patients with acute SCI and found that patients with complete motor loss had higher levels of NF-L than those with partial motor loss, and NF-L levels in the CSF of patients without neurological improvement were 10 times higher than those in patients with improvement, indicating that SCI could be diagnosed based on CSF levels of NF-L. In a subsequent study with a larger sample (23 SCI patients and 67 healthy controls). Kuhle et al. (2015) confirmed that baseline serum NF-L levels were significantly higher in patients with motor-complete SCI (70 pg/mL) and motor-incomplete SCI (21 pg/mL) than in healthy controls (5 pg/mL), and serum NF-L was significantly correlated with ASIA grade and motor score at baseline, 24 hours, and 3–12 months. They also showed that minocycline treatment reduced serum NF-L levels in the subgroup of patients with motor-complete SCI, suggesting that NF-L may help to guide treatment, as well as being a diagnostic and prognostic indicator (Kuhle et al., 2015).

Other Structural Biomarkers for Spinal Cord Injury

Tau, as the most abundant tubule-associated protein, contributes to microtubule formation and stabilization inside neuronal cells. Kwon et al. (2010) showed that CSF tau levels increased in a severity-dependent manner among patients with AIS grades A, B, and C, and that a lower tau level predicted an improvement of one AIS grade at 6 months after SCI injury (Kwon et al., 2017). Dalkilic et al. (2018) confirmed that CSF tau levels differed significantly among SCI patients with AIS grades A, B, and C, and motor score improvement 6 months later was best predicted by structural proteins, such as CSF tau. A recent study demonstrated that CSF and serum tau levels peaked rapidly at 12 hours after injury in a severity-dependent SCI rat model, and Basso, Beattie, and Bresnahan locomotor rating scale scores were positively linearly correlated with tau protein levels (Tang et al., 2019). Because tau is hyperphosphorylated under conditions of pathological axonal damage, such as in TBI (Rubenstein et al., 2015), recent studies have examined the potential role of hyperphosphorylated tau (p-tau) as a biomarker for SCI. Caprelli et al. (2018) proved that the presence of p-tau was in line with the temporal and spatial distribution of axonal damage in SCI model rats, and CSF and serum levels of p-tau were greatly increased after SCI. However, there are currently no clinical data regarding dynamic changes in p-tau in biofluids in SCI patients, and the relationship between p-tau protein and AIS grading remains unknown. Overall, evidence suggests that total tau can serve as a diagnostic and prognostic biomarker for SCI, while p-tau may also have a certain diagnostic value for SCI.

Matrix metalloproteinases (MMPs) comprise a large family of zinc-bound proteases with strong effects on the degradation of protein components in the extracellular matrix. Moghaddam et al. (2017) analyzed the data sets for 115 patients with traumatic SCI (33 women, 82 men) published from 2010 to 2015, including serum MMP levels at different time points from admission to 12 weeks after injury. They found that MMP-8 and MMP-9 levels differed significantly between SCI patients and controls, and the favored predictive model consisting of MMP-8 levels 1 day and MMP-9 levels 1 month after injury could predict neurological remission in 97% of cases (Moghaddam et al., 2017).

Inflammatory Biomarkers for Spinal Cord Injury

Damaged tissues and a ruptured blood-spinal cord barrier following SCI lead to the accumulation of pro-inflammatory

cytokines and chemokines in the CSF or peripheral blood, enabling these molecules to serve as latent biomarkers. Among the inflammatory mediators, interleukins have been studied extensively in clinical research and have shown great potential as SCI biomarkers. Davies et al. (2007) conducted a cross-sectional study of 56 SCI patients and 35 age-matched controls, and found that serum interleukin-6 (IL-6) and IL-1 levels were significantly higher in SCI patients than in controls at 2–52 weeks post-injury; however, increased cytokine levels were not correlated with injury level or AIS classification. However, Bank et al. (2015) found that serum IL-6 levels were significantly elevated in patients with acute SCI, but only in the first week of injury. Similarly, de Mello Rieder et al. (2019) reported that serum IL-6 levels were only increased 48 hours post-injury, and there was no correlation between IL-6 levels and short- or long-term prognosis (i.e., survival rate or sensory and motor function improvement).

CSF levels of interleukins differ from plasma levels in patients with SCI. Kwon et al. (2010) demonstrated that CSF IL-6 and IL-8 increased in severity-dependent manners in SCI patients, and IL-8, together with S100β and GFAP, was used to establish a biochemical model that could predict ASIA grade with 89% accuracy after 24 hours of injury. Furthermore, these CSF proteins were more predictive of segmental motor recovery than baseline ASIA grade at 6 months after injury. Kwon et al. (2017) also verified that CSF IL-6 expression levels differed significantly among patients with acute SCI with baseline ASIA grades of A, B, and C. Moreover, IL-6 was strongly correlated with ASIA conversion and motor improvement 6 months after SCI. In another clinical study comparing CSF and MRI biomarkers for acute SCI, Dalkilic et al. (2018) showed that CSF IL-6 and IL-8 levels were correlated with baseline ASIA grades. In addition, CSF levels of inflammatory cytokines (IL-6, IL-8, monocyte chemotactic protein-1) performed best for predicting ASIA grade conversion, while structural biomarkers (tau, GFAP, S100β) performed best for predicting motor score improvement.

Other inflammatory cytokines, such as tumor necrosis factor α (TNFα) and IL-1β, which are secreted mainly by monocytes or macrophages, also participate in the secondary inflammatory response in SCI. Hayes et al. (2002) showed that serum TNFα levels were significantly higher in patients with chronic SCI (>12 months) compared with normal subjects. In addition, Davies et al. (2007) found that higher serum TNFα levels were sustained in SCI patients from 2–52 weeks post-injury, and TNFα levels differed among patients with different ASIA classifications. However, Biglari et al. (2015) found that serum TNFα levels were significantly lower in patients with AIS improvement at 9 hours after SCI than in those without. Meanwhile, serum IL-β levels exhibited a stable decline from 2–12 weeks in SCI patients, despite showing no distinction in relation to ASIA conversion. A recent study by Ogurcov et al. (2021) confirmed that serum TNFα levels differed between patients with ASIA grades A and B. In another study based on 140 participants, Xu et al. (2015) revealed that SCI patients with neuropathic pain had significantly higher serum TNFα levels. In conclusion, serum TNFα could play a part in the diagnosis of SCI, classification of ASIA grade, and prognosis of neurological improvement, and dynamic measurement of serum IL-1 might be useful for tracking the progression of SCI.

Other cytokines, such as growth factors, have also proven useful for the assessment of patients with SCI. Ferbert et al. (2017) found that serum levels of insulin-like growth factor 1 (IGF-1) and transforming growth factor-β (TGF-β) were both elevated during the acute and sub-acute phases after injury in 23 SCI patients, and higher serum IGF-1 levels predicted insufficient neurological remission 12 weeks later, while TGF-β showed no such effect. However, a study of 45 SCI patients by Moghaddam et al. (2016) drew different conclusions, and found that high serum IGF-1 levels were related to neurological recovery. Information on the relationships between these cytokines and AIS classification is also lacking, and more studies are needed.

Spinal Cord Injury RNA Biomarkers

Studies of non-coding RNA (ncRNA) sequences after SCI are shown in **Table 2**.

Table 2 | **Non-coding rnas sequencing after SCI**

Category	Reference	Method	Species	Group	Sample size (n)	Bio-sample	Time of sampling	Key mirnas	Role in SCI
miRNAs	Hachisuka et al., 2014	Taqman low-density array analysis	Mouse	Normal (without surgery); sham (laminectomy only); mild (50 kdyn); severe (70 kdyn)	1	Serum	12 h after SCI	mir-9*, mir-219 and mir-384-5p	Positively related with SCI severity
	Tigchelaar et al., 2017	Next-generation sequencing	Female Yucatan miniature pigs	Sham (laminectomy only); mild (10 cm of 50 g weight); moderate (20 cm of 50 g weight); severe (40 cm of 50 g weight)	4	CSF and serum	15 min before injury; 1, 3, and 5 d after injury	58, 21, 9 and 7 altered mirnas were identified in severe, moderate, mild SCI and sham surgery groups	Overall mirna expression at 1 and 3 d post injury was strongly correlated with SCI outcome
	Ding et al., 2019	Next-generation sequencing	Rat	Sham (laminectomy only); SCI (120 kdyn)	3	Serum exosomes	6 h after injury	mir-125b-5p, mir-152-3p, and mir-130a-3p	Acute SCI diagnosis
	Ding et al., 2020	Next-generation sequencing	Rat	Sham (laminectomy only); SCI (120 kdyn)	3	Serum exosomes	7 d after injury	mir-485, mir-30b, mir-26b, mir-23b, mir-223, mir-21, mir-211, mir-200c, mir-195, mir-17, mir-133a, mir-125b-1, mir-124, mir-103, mir-672 and mir-15b	Subacute SCI diagnosis
LncRNAs	Ding et al., 2016	Microarray analysis	Mouse	Five groups (sham operation, 1 3 d after injury, 1 and 3 wk after injury)	3	Spinal cord	At 1 and 3 d, 1 and 3 wk after injury	345, 502, 891, and 181 lncRNAs were differentially expressed at 1, 3, 7, and 21 d after injury; 458, 1073, 2177 and 850 mRNAs were differentially expressed at 1, 3, 7, and 21 d after injury	Lncrnas and mirnas expression peaked 1 wk after SCI
	Duran et al., 2017	RNA sequencing	Rat	Four groups (sham operation, 1, 3 and 6 mon after SCI), 150 kdyn	3	Spinal cord	At 1, 3 and 6 mon after injury	2935, 3981 and 3206 protein-coding genes were differentially expressed at 1, 3 and 6 mon after injury; 137, 239 and 179 lncRNAs was differentially expressed at 1, 3 and 6 mon after injury.	Sub-chronic and chronic stages of SCI
	Zhou et al., 2018	Microarray analysis	Rat	Control (without surgery); SCI group (10 g × 50 mm)	9	Spinal cord	2 h after SCI	772 lncRNAs and 992 mRNAs were differentially expressed	Immediate phase of SCI
	Shi et al., 2019	Microarray analysis	Rat	Control (without surgery); SCI group (10 g × 50 mm)	9	Spinal cord	2 d after SCI	3193 lncRNAs and 4308 mRNAs were differentially expressed	Early acute phase of SCI

1 dyn = 10^{-5} N. CSF: Cerebrospinal fluid; lncRNAs: long-coding RNAs; miRNAs: microRNAs; SCI: spinal cord injury.

Review

Use of microRNAs as biomarkers for SCI

Single-stranded ncRNA molecules with a length of 22 nucleotides, known as microRNAs (miRNAs), participate in the regulation of post-transcriptional gene expression. Differentially expressed miRNAs have been shown to contribute to pathological reactions (including apoptosis, inflammatory response, angiogenesis, etc.) in SCI, making them potential therapeutic targets for SCI intervention (Nieto-Diaz et al., 2014; Shi et al., 2017). Hachisuka et al. (2014) established a mouse SCI model and found that serum expression levels of miR-9*, miR-219, and miR-384-5p were significantly increased in the normal, sham, mild, and severe groups in a severity-dependent manner at 12 hours after injury. Tigchelaar et al. (2017) evaluated serum miRNA levels at 1, 3, and 5 days after injury in a porcine model of SCI, and quantified seven, nine, 21, and 58 differentially expressed miRNAs in the sham surgery, mild, moderate, and severe injury groups, respectively. They also found that SCI functional outcome was significantly correlated with serum miRNA levels at 1 and 3 days after injury, supporting a role for serum miRNAs as promising biomarkers for the assessment of SCI severity and future functional amelioration.

Although circulating miRNAs have potential as biomarkers in SCI, their concentration and stability in body fluids, such as blood and CSF, are limited. However, exosomes are lipid-bilayer-encapsulated particles containing various proteins and miRNAs that are released from cells into the extracellular fluid, and exosomal miRNAs have exhibited greater effects in terms of the pathobiology and therapy of SCI than free miRNAs (Dutta et al., 2021). Ding et al. (2019) analyzed miRNA profiles in serum exosomes at 6 hours after injury in a rat SCI model, and found that serum exosomal miR-125b-5p, miR-152-3p, and miR-130a-3p had sufficient specificity and detectability to act as diagnostic biomarkers for acute SCI. They also confirmed that 141 miRNAs in serum exosomes were differentially expressed between SCI and sham rats at 7 days after injury, showing potential diagnostic and prognostic values for subacute SCI (Ding et al., 2020). Overall, circulating miRNAs, especially exosomal miRNAs, could play a vital role as potential biomarkers for SCI.

Use of long non-coding RNAs as biomarkers for SCI

As a class of ncRNA molecules with more than 200 nucleotides, long non-coding RNAs (lncRNAs) are involved in many cellular activities, including epigenetic regulation, the cell cycle, and differentiation regulation. These lncRNAs have been a recent research hot spot, and they have been confirmed to be related to various diseases such as cancer and nervous system diseases (Quan et al., 2017), including SCI (Wang et al., 2019). Studies in rats and mice have revealed a relationship between lncRNAs and SCI (Wang et al., 2019). Ding et al. analyzed the lncRNA profile of the spinal cord in an SCI mouse model and found differential expression of lncRNAs from 1 day after injury, reaching a peak at 1 week and then decreasing until 3 weeks after SCI (Ding et al., 2016). Duan et al. (2017) found that the expression profile of spinal cord lncRNAs changed dynamically during the chronic phase of SCI (1, 3, and 6 months). In another study using SCI rat models, Shi et al. (2019) quantified 3193 differentially expressed lncRNAs and 4308 differentially expressed miRNAs at 2 days after injury, while regarding the immediate phase of SCI, Zhou et al. (2018) identified 772 differentially expressed lncRNAs and 992 differentially expressed miRNAs at 2 hours after injury in a rat model. In a weighted gene co-expression network analysis, Chen et al. (2021) identified six genes (CCNB2, CCNB1, CKS2, COL5A1, KIF20A, and RACGAP1) that could act as potential biomarkers to differentiate among SCI subtypes. However, despite the use of animal models to reveal the specific molecular mechanisms of several lncRNAs in the pathogenesis and progression of SCI (Jiang and Zhang, 2018; Zhang et al., 2018; Guan and Wang, 2021), clinical data regarding the potential role of circulating lncRNAs as biomarkers for the diagnosis or prognosis of SCI are extremely limited. The current findings provide a promising basis for future research into the role of lncRNAs in SCI.

Trace Elements as Prognostic Biomarkers for Spinal Cord Injury

Oxidative stress and inflammatory responses are critical factors in the occurrence of secondary injury during the pathological progression of traumatic SCI. Essential trace elements might play a protective role as antioxidants during this process, and may thus have a close relationship with future neurological recovery. Sperl et al. (2019) studied 29 patients with acute traumatic SCI and found that patients without neurological remission (G1) had significantly higher serum Mg levels than patients with neurological remission (G1) within the first 7 days. Heller et al. (2019) and Seeling et al. (2020) found that patients with traumatic SCI with neurological remission (G1) who had a higher serum Se levels on admission exhibited a significantly more rapid decline within the first week compared with patients without neurological improvement, with a similar effect for serum Cu. However, regarding Zn levels, Kijima et al. (2019) reported that a dramatic decrease in serum Zn levels was related to the severity of SCI, and Zn levels 12 hours after injury could predict neurological functional recovery in a mouse model. In addition, they demonstrated that the severity-dependent decrease in serum Zn was due to a proportional increase in Zn uptake by circulating activated monocytes. Heller et al. (2020) also claimed that early changes in serum Zn levels from 0–9 hours after SCI had an accuracy of 82.2% for predicting neurological impairment. In brief, changes in serum levels of essential trace elements may reflect the consistent redistribution of these antioxidants, and temporal fluctuations in these microelements might thus have predictive value in terms of neurological regeneration (**Table 3**).

Limitations

At present, although many potentially effective biomarkers have been discovered, few clinical studies have been conducted to determine which biomarkers are most closely related to the severity and prognosis of SCI, which is a major issue in their clinical application. This review did not consider all the possible biomarkers, and only listed those with the highest correlations with SCI. Regarding the acquisition of repeated CSF samples, careful attention must be paid to aseptic procedures and to ensuring an adequate time interval between samples.

Conclusion

Biomarkers should ideally possess as many of the following properties as possible: (1) changes (preferably increases) in CSF, serum, plasma, or whole-blood levels after SCI, with low levels in normal people; (2) levels in biological fluids easy to measure and quantify using sandwich enzyme-linked immunosorbent assays or similar immunoassays, with at least two detection formats or platforms; (3) ability to measure biomarker levels repeatedly within a 48-hour window after SCI; and (4) levels of the biomarker in the biofluid should respond to treatment (Wang et al., 2018).

According to the current summary, GFAP, S100β, UCH-L1, NF-L, tau, IL-6/8, TNFα, and Zn could be useful diagnostic and prognostic biomarkers, and indicators of AIS classification in SCI; NSE, pNF-H, and miRNAs have possible roles in the diagnosis and severity assessment of SCI; IGF-1 and MMPs may be involved in the diagnosis and neurological recovery of SCI; TGF-β and lncRNAs are only useful for the recognition of

Table 3 | Summary of trace element in SCI

Reference	Phase of SCI	Testing method	Biomarker	Groups and sample size	Type of sample	AIS grade	Time of sampling	Role in prediction of SCI recovery	Conclusion
Heller et al., 2019	Acute, subacute and intermediate	Total reflection X-ray fluorescence and ELISA	Selenium Selenoprotein P (SELENOP)	39 TSCI patients (group C: 10 patients without neurological impairment; group G_0: 9 patients without remission; group G_1: 10 patients with remission-AIS conversion within 3 mon)	Serum	A–C, E	On admission, at 4, 9 and 12 h, 1 and 3 d, 1 and 2 wk, and 1, 2 and 3 mon	Binary logistic regression analysis including the delta of Se and SELENOP within the first 24 h indicated an area under the curve (AUC) value of 90.0% (CI: 67.4–100.0%) with regards to predicting the outcome after TSCI	A Se deficit might constitute a risk factor for poor outcome after TSCI
Kijima et al., 2019	Acute and subacute	Metallo assay Zn LS Kit	Zn	38 SCI patients	Serum	A–D	Within 3 d after SCI	Serum zinc concentrations in the acute-phase accurately predicted the long-term functional outcome (R^2 = 0.84)	The acute-phase serum zinc concentration can be a useful biomarker for predicting the functional prognosis
Sperl et al., 2019	Acute, subacute and intermediate	ADVIA chemistry XPT system by Siemens Healthineer®	Magnesium	29 patients were divided into two groups (group G_0: 11 patients without remission; group G_1: 10 patients with remission-AIS conversion within 3 months)	Serum	A–D	On admission, and at 4, 9 and 12 h, 1 and 3 d, 1 and 2 wk, and 1, 2 and 3 mon	At 7 d, Mg concentrations in G_1 and G_0 were significantly different ($G_0 > G_1$). Significant differences were detected between patients in G_1 that presented an AIS (ASIA Impairment Scale) conversion of 1 level versus those with more than 1 level (G_1 AIS imp. =+1 > G_1 AI imp. > +1).	Low and decreasing levels of Mg within the first 7 d are indicative of a high probability of neurological remission, whereas increasing levels are associated with poor neurological outcome
Heller et al., 2020	Acute and subacute	Total reflection X-ray fluorescence (TXRF)	Zn	42 patients with TSCI [two groups: a study group S (n = 33) with neurological impairment and a control group C (n = 9) without neurological impairment]	Serum	A–C	On admission, and at 4, 9, 12 and 24 h, and 3 d after SCI	In comparison to the initial Zn Concentration, the AIS imp > + 1 patients reached their minimum of 58% at 4 h after trauma, whereas patients with an AIS imp = +1 reached their minimum of 56% at 9 h after TSCI	Early Zn concentration dynamics differed in relation to the outcome and may constitute a helpful diagnostic indicator for patients with SCI
Seelig et al., 2020	Acute	Total reflection X-ray fluorescence and ELISA	Selenium, Copper, Selenoprotein P (SELENOP), Ceruloplasmin (CP)	52 TSCI patients (G_1: 21 patients with AIS conversion within 3 mon; G_0: 21 patients without AIS conversion within 3 mon; control: without neurological impairment)	Serum	A–C	On admission, and at 4, 9, 12, and 24 h after injury	Binary logistic regression analysis including Cu and Se levels on admission in combination with Se and CP levels at 24 h after SCI allowed a prediction for potential remission	These data indicate a strong association between temporal changes of the Se and Cu status and the clinical outcome after TSCI

AIS: American Spinal Injury Association Impairment Scale; SCI: spinal cord injury; TSCI: traumatic SCI.

SCI; and Mg, Se, and Cu are mainly related to the prognosis of SCI (**Figure 2**).
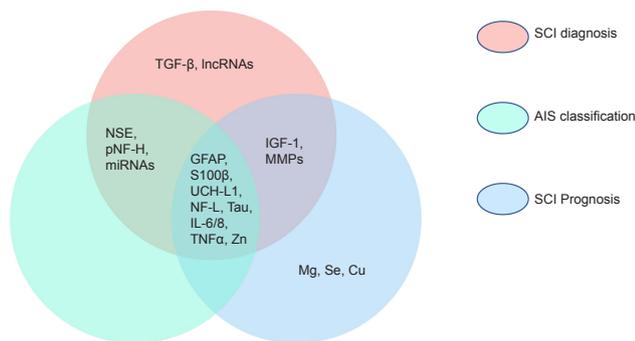

Figure 2 | Venn diagram of current research results for potential biomarkers of spinal cord injury (SCI).
AIS: American Spinal Injury Association Impairment Scale; GFAP: glial fibrillary acidic protein; IGF-1: insulin-like growth factor-1; IL: interleukin; lncRNA: long non-coding RNA; miRNA: microRNA; MMP: matrix metalloproteinase; NSE: neuron-specific enolase: pNF-H: phosphorylated neurofilament-heavy; TGF-β: transforming growth factor-β; TNFα: tumor necrosis factor α; UCH-L1: ubiquitin C-terminal hydrolase-L1.

We assume that the combination of two or three of these recently identified biomarkers may have useful potential in the diagnosis, prognosis, and even treatment of SCI. However, further, high-quality prospective clinical studies are needed to rigorously analyze the sensitivity, specificity, and predictability of the available biomarkers.

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