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Potential diagnostic and prognostic value of serum and cerebrospinal fluid biomarkers in traumatic spinal cord injury: A systematic review

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List of abbreviations:

AIS	American Spinal Injury Association Impairment Scale
CCL	chemokine (C-C motif) ligand
CCS	Case-control study
Cross	Cross sectional
CS	Cohort study
CSF	Cerebrospinal fluid
CXCL	Chemokine (C-X-C motif) ligand
GFAP	Glial fibrillary acidic protein
HGF	Hepatocyte growth factor
HMGB1	High mobility group box 1 protein
IGF-1	Insulin-like growth factor 1
IL	Interleukin
INF-γ	Interferons-γ
IP-10	Inducible protein-10
MCP	Monocyte chemotactic protein
MIF	migration inhibitory factor
MMP	Matrix metalloproteinase
NA	Not applicable
NFH	Neurofilament heavy chain
NF-L	Neurofilament light chain
NOx	Nitric oxide
NR	Not reported

NSE	Neuron specific enolase
RCS	Retrospective cohort study
sCD95L	Serum cluster of differentiation 95 ligand
SCGF-β	Stem cell growth factor beta
SCI	Spinal cord injury
TGF- β1	Tumour growth factor β1
TNF-α	Tumour necrosis factor-α

Abstract

It remains unclear whether biomarkers in the serum or cerebrospinal fluid (CSF) can be used for diagnosis or prognosis of spinal cord injuries (SCI). Therefore, a systematic review was undertaken to evaluate the prognostic or diagnostic value of serum and CSF biomarkers in assessing the severity of SCI and the outcome of patients. Two independent reviewers summarized the human studies retrieved from the electronic databases of Medline, Embase, Scopus and ISI Web of Science until April 2018. Seventeen studies were included (1065 patients aged 16 to 94 years old). Although the findings of the included studies suggest that inflammatory and structural proteins may be useful in assessing the severity of SCI and prediction of neurological outcome, the level of evidence is generally low. Given limitations to the available evidence, further investigation in this field is required using large prospective datasets with rigorous analysis of sensitivity, specificity and prediction.

Keywords: Spinal cord injuries; Biomarkers; Prognostics value

1.1 Introduction

Spinal cord injury (SCI) is one of the most serious injuries that can severely affect a person's function. The incidence of SCI has been reported at 10.5 cases per 100,000 people (1). Epidemiological studies conducted in the last decade have clearly shown that SCI mostly affects younger adults (average age of 34.0 to 39.8 years old) (1, 2). No effective treatment has been introduced that can significantly improve sensory and motor function in SCI patients (3); however, considerable improvements have been made in secondary care of these patients that have led to a decrease in their mortality rates (4).

After primary stabilization of patients in the first few days after a SCI, the patients and their families want to know whether they can return to their normal independent lives or not (5). Therefore, a correct assessment of the severity of the SCI is of utmost importance for predicting the functional outcome of the patients. Currently, SCIs are classified according to the American Spinal Injury Association (ASIA) Impairment Scale (AIS), which is a revised version of Frankel criteria (6). Although the AIS criteria is the most commonly used tool for classification SCIs, it has some limitations (7) that have led researchers to search for other auxiliary tools for accurate assessment of the patients' status such as magnetic resonance imaging (MRI) (8-10), electrophysiological evaluations and biomarker measurement in the serum and cerebrospinal fluid (CSF) (11-15). Biomarkers are excreted into serum and CSF during various stages of an SCI (16, 17) and they include inflammatory factors and structural proteins such as S100 calcium-binding protein β (S100-β), Tau protein and neuron specific enolase (NSE) (18-21). Current evidence raises the question of whether these biomarkers could be used as a tool for an accurate classification of SCI or not, for which no concrete answer has been established. Hence, the present systematic review has aimed to gather the

findings of all the studies that have assessed the predictive or diagnostic value of serum or CSF biomarkers in detecting the severity of SCI and the outcome of affected patients, in search of a consensus regarding this question.

1.2 Methods

1.2.1 Study design

The present study was designed to investigate the diagnostic value of serum and CSF biomarkers through a systematic review. The study was carried out according to the guideline for the systematic reviews and meta-analyses in observational studies (MOOSE) guideline (22).

1.2.2 Search strategy

A search was performed in the electronic databases and the bibliographies of relevant articles. Search in grey literature was also carried out as another source of possible related studies. The systematic search of electronic databases was conducted with the guidance of a librarian and under supervision of an experienced researcher in the field of SCI. Keywords for the search were selected according to the Mesh database and Emtree, after consultation with a neurosurgeon and review of the titles and abstracts of relevant articles. Then, the search strategy was defined for each database, according its specific guides. Further details on the search and data summarization methods can be found in the previous meta-analyses of the authors (23, 24). The electronic databases of Medline, Embase, Web of Science and Scopus were searched until April 2018. The search strategy for the Medline database is presented in Table 1 as a template.

1.2.3 Selection criteria

The human studies investigating the diagnostic value of serum and CSF biomarkers and their predictive value for the patients' outcomes were included in the present study. Lack of a control group or a group without any changes in neurological status in follow up, inclusion of chronic or non-traumatic injuries and not describing the protocol used for measurement of the biomarker were considered as the exclusion criteria.

1.2.4 Data collection

Two independent reviewers performed screening, summarization and the quality control of the included articles. Any disagreement was resolved through discussion with a third person. Articles were summarized based on a checklist designed according to the PRISMA statement guidelines (25). Extracted information included data related to the methods of the study, characteristics of the case and control groups (age, gender and SCI mechanism), number of included cases, outcome and possible biases. Diagnostic value of the biomarker in detection of SCI and its prognostic value for neurological improvement were the outcomes evaluated in the study. The plot digitizer software (version 2.0; available in: http://plotdigitizer.sourceforge.net) was used to extract the information from the articles that presented their results as charts.

1.2.5 Quality control of the studies

Quality assessment of the articles was performed per QUADAS-2 guidelines (26). Inter-rater reliability was evaluated to determine the agreement between the two reviewers. Disagreements were resolved through discussion with a third researcher.

1.3 Results

1.3.1 Characteristics of the articles

The systematic search yielded 1072 non-repetitive articles. The primary screening downsized this number to 56 potentially relevant studies, and eventually after review of these articles' full texts, 16 studies were included (7, 20, 27-40) (Figure 1). These papers included data from 1031 subjects (age range of 16 to 94 years). Seven studies were cohort (7, 20, 34-36, 38, 40), seven were case-control (27, 28, 31-33, 37, 39) and two were cross-sectional (29, 30). The severity of injury ranged from A to D, according to the AIS. The duration between injury and biomarker measurement varied from zero to 90 days.

Evaluated biomarkers were categorized into three groups of inflammatory factors, structural proteins and others (nitric oxide, stem cell growth factor beta and hepatocyte growth factor). Inflammatory factors included interleukins (ILs), chemokines and other cytokines. Structural proteins included Tau protein, S100-β protein, glial fibrillary acidic protein (GFAP), matrix metalloproteinases (MMPs), neurofilaments, high mobility group box 1 protein (HMGB1) and NSE. Tables 2 and 3 present a summary of the included articles.

Of these 16 studies, 10 had evaluated the diagnostic value of the aforementioned biomarkers for SCI (20, 27, 28, 31, 33-35, 37, 39, 40) and seven focused on their prognostic value (7, 29, 30, 32, 36, 38, 39) for neurological improvement/remission.

1.3.2 Quality control of the studies

Quality assessment of the articles showed that patient selection of 62.5% and 18.8% of eligible studies have high and unclear risk of bias, respectively. Moreover, risk of bias for index test was unclear in 68.8% of the studies. Only 6.2% of the articles had unclear risk of bias for reference standard, while 31.2% and 6.2% of flow and timing of included studies had proposed high and unclear risk of bias, respectively. The applicability of patient selection was also at high risk in 12.5% of the studies (Table 4 and Figure 2).

1.3.2.1 Diagnostic value of serum and CSF biomarkers for SCI

Ten studies had evaluated the diagnostic value of serum and CSF biomarkers for SCI (20, 27, 28, 31, 33-35, 37, 39, 40). These biomarkers included various cytokines (ILs, chemokines and other cytokines) and structural proteins (MMPs, GFAP, S100-B, NSE, neurofilaments, Tau protein, and HMGB1) (Table 2).

a) Serum level of biomarkers in diagnosis of SCI

- **Diagnostic value of ILs for SCI**

The included studies investigated the value of IL-1, IL-5, IL-6, IL-9, IL-10, IL-17, IL-16 and IL-18 in diagnosis of SCI. The findings of this section are indicative of a significant change in the serum concentration of these ILs after SCI. For instance, Zaaqoq et al. reported significant decreases in the serum level of IL-1β on days 1, 4, 7, 9, 13 and 14 after SCI, while no considerable difference is appreciated between the case and control groups on other days. A similar pattern was reported for IL-5, with its levels significantly lower in the case group on the first four days after SCI, and on days 7, 9 and 14 (40).

Bank et al. also showed that the serum level of IL-6 is significantly higher in patients with SCI, on the first 3 days, days 4 to 7 and day 14 after the injury (28). On the contrary, Zaaqoq et al. reported no considerable changes in IL-6 levels in the first 14 days after the injury. These two studies showed a significant increase in levels of IL-9, IL-10, IL-16 and IL-18 in the first days following SCI along with a significant decrease in levels of IL-13 and IL-17, compared to the control group (28, 40).

Overall, these findings are shown a significant change in the levels of ILs after SCIs, which renders them suitable candidates for diagnosis of these injuries (Table 5).

- **Diagnostic value of chemokines for SCIs**

Chemokines are other factors that show increased concentrations after SCI. Included articles had assessed the value of chemokine (C-X-C motif) ligand (CXCL)-1, CXCL-2, CXCL-12, chemokine (C-C motif) ligand (CCL)-4, monocyte chemotactic protein (MCP)-l and inducible protein-10 (IP-10) in diagnosis of SCIs.

According to Bank et al. study the circulating level of CXCL-1 is significantly higher in SCI patients compared to healthy controls during the first week after the injury, and on days 11 to 14 (28). In another study, Hassanshahi et al. reports this level to be only significantly increased on day 7 after the SCI. CXCL-9 is another biomarker with increased concentrations in patients with SCI on day 7 after their injury. The levels of CXCL-10 and CXCL-12 also rise in the first week after injury and could stay at a high level until day 28 (31).

The results of the studies that had evaluated the serum levels of CCL-4 were contradictory, with Bank et al. reporting a significant increase in its level after SCIs, while Zaaqoq et al. found a significant drop in SCI patients (28, 40) (Table 5).

- **Diagnostic value of other cytokines for SCI**

SCI affects the serum levels of migration inhibitory factor (MIF) and interferon gamma. Zaaqoq et al. reported a significant drop in serum levels of interferon gamma immediately after SCI and during the first week after it, compared to healthy controls (40). In the second week, the differences between the two groups are not significant and the concentrations return to normal levels. Bank et al. found a significant rise in MIF levels of patients with SCI; however, this increase is only observed on day 7 after the injury, and it returns to normal afterwards (28).

- **Diagnostic value of structural proteins for SCI**

As a result of injury in central nervous system, structural proteins are released into the serum. Increased levels of these biomarkers could be valuable for detection of SCI. In 2017, Moghaddam et al. reported a significant rise in MMP-8 serum levels in the first 48 hours after SCI, while no significant changes in concentrations of MMP-9 and MMP-2 were observed (35). GFAP is another structural protein that was investigated by Ahadi et al., who found a prominent increase in its levels within the first 48 hours, followed by a quick return to base levels after 72 hours. These researchers report a similar trend for the heavy subunit of neurofilaments, with its concentrations increasing within the first 48 hours after an injury and a return to that of the healthy group after 72 hours (27). Serum levels of neurofilament light chain after complete and incomplete SCIs were also reported by Kuhle et al. to be

significantly higher in the case group compared to the control group in the first week after the injury (34).

Two studies assessed the value of NSE for diagnosis of SCI. In the first one conducted by Wolf et al. in 2014, no considerable difference was observed in the level of this biomarker between the two groups of case and control within the first 24 hours after the injury (20). On the other hand, in 2015, Ahadi et al. reported a significant increase in NSE's serum levels in the first 48 hours after SCI, which tends to return to normal levels by the third day (27). Wolf et al. also assessed the changes in S-100β levels and found no considerable changes after SCI (20). In addition, serum concentration of HMGB1 showed a statistically significant raise in SCI patients (37) (Table 5).

b) CSF level of biomarkers in diagnosis of SCI

Two studies assessed the diagnostic value of CSF level of heavy subunit of neurofilaments (39) and nitric oxide (33) in detection of SCI. Ungureanu et al. confirmed a substantial increase in CSF levels of heavy subunit of neurofilaments during the first three days between SCI patients and the control group (39). However, Hoska et al depicted nitric oxide levels did not differ significantly between SCI patients and uninjured controls (30).

1.3.2.2 Prognostic value of serum and CSF biomarkers for neurological improvements/remission

The prognostic value of serum and CSF biomarkers for neurological improvements/remission had been evaluated in 7 studies (7, 29, 30, 32, 36, 38, 39). The biomarkers in this section were categorized into two groups of cytokines and structural proteins (Table 6).

a) Serum level of biomarkers in prognosis of SCI

- **Prognostic value of IL1-β**

Only one study evaluated the prognostic value of serum level of IL-1β in SCI (29). In this regard, Biglari et al. found IL-1 to have no prognostic value for neurological improvement after an SCI (Table 6).

- **Prognostic value of chemokines**

The studies included in this section had assessed the prognostic value of CCl-2, CCl-3, CCL-4, and CXCL-5 for neurological status of the patients. Heller et al. also found the levels CCL-2 and CCL-4 to be significantly lower in the first and 9 hours after admission, in patients who went through neurological improvements, while no considerable changes were reported for the levels of and CCL-3 and CXCL-5 (32).

The serum levels of tumour necrosis factor – α (TNF- α) were also reported by Biglari et al. to be significantly lower at hour 9 after the SCI, in patients who had neurological improvements compared to other SCI subjects (12-week follow up) (29). These researchers

observed no significant differences in TNF- α levels between the two groups at other time points (Table 6).

- **Prognostic value of other cytokines**

Growth factors such as insulin-like growth factor 1 (IGF-1) and tumour growth factor β1 (TGF- β1) were two of the most commonly assessed chemokines in regards to prognosis of SCI. Febert et al. showed that TGF- β1 is not a suitable prognostic factor for SCI patients (30). However, they found a considerable rise in levels of serum cluster of differentiation 95 ligand (sCD95L) on day 7 after an injury in patients who had neurological improvement. This change was transient and the concentration of this chemokine lowered back to that of the subjects with no neurological improvements. As for the IGF-1, in another study conducted by Moghaddam et al., this chemokine was reported to rise in the subacute and chronic phases (days 7, 14, and 56 post-SCI) in the patients with neurological improvement (36).

- **Prognostic value of structural proteins**

Moghaddam et al. have suggested that serum levels of MMP-8 in the first 24 hours after injury could be good prognostic marker for perdition of neurological outcome of the patients. The serum concentration of this biomarker was found to be significantly lower in patients with neurological remission compared to other patients, but no considerable changes were appreciated in the levels of MMP-2 and MMP-2 (35).

Kuhle et al. refers to neurofilament light chain as a prognostic marker for neurological improvement in SCI patients. Their results show a significant increase in the serum levels of this biomarker in the first 24 hours, which stays at a high level until one week after the injury. The concentration was found to be much higher among patients with poor outcomes (34) (Table 6).

b) CSF level of biomarkers in prognosis of SCI

- **Prognostic value of ILs**

Kwon et al. reported the mean concentrations of IL-6 and IL-8 in the CSF to be significantly lower in the first 24 hours after injury, among patients with neurological improvements compared with subjects with no changes in neurological status (7) (Table 6).

- **Prognostic value of chemokines**

One study included in this section had assessed the prognostic value of MCP-1 for neurological status of the patients. Kwon et al. reported the CSF level of MCP-1 to be significantly lower in patients who showed neurological improvements, compared to the rest of the patients (7) (Table 6).

- **Prognostic value of structural proteins**

The prognostic value of CSF level of neurofilaments had been investigated in two of the articles. Pouw et al. argued that the CSF levels of neurofilament heavy chain in the first 24 hours after SCI cannot be a useful prognostic factor for neurological outcome of these

patients (38). In another study with a 12 to 18-months follow up of patients, Ungureanu et al. showed that this biomarker is able to predict the outcome of patients when measured in the first 6 hours, but its levels do not show a significant difference when measured after 24 hours (39).

Two of the studies included in our review measured the CSF levels of GFAP in the first 24 hours after SCI. Kwon et al. reported higher levels of GFAP in patients with no neurological improvements within 6 months of the injury (7). In addition, Pouw et al. found a significant correlation between the CSF levels of GFAP with neurological remission in SCI patients (38).

S100-β, Tau protein and NSE also increase in SCI patients and this rise could be correlated with the severity of injury (7, 38). Kwon et al. confirmed that CSF levels of S100-β and Tau protein is significantly higher in the first 24 hours after SCI in patients with no neurological improvement compared to other cases. However, Pouw et al. reported no significant difference in the concentration of NSE between the two-mentioned group of patients (38) (Table 6).

1.4 Discussion

The present systematic review collected available evidence on the value of serum and CSF biomarkers in diagnosis of SCI and prognosis of neurological improvement in affected patients. This study showed that overall, the concentration of inflammatory factors and structural protein changes in the serum and CSF in response to an SCI. After the injury, the

serum and CSF levels of IL-1β, IL-5, and IL-17 drop while the concentration of IL-6, Il-10, IL-16, IL-18, CXCL-1, CXCL-9, CXCL-10, CXCL-12, and MIF increases. There is disagreements between the studies regarding the changes in the levels of CCL-4. A significant rise also occurs in the levels of the structural proteins MMP-8, GFAP, neurofilaments, NSE, S100-β and HMGB1.

Changes in the levels of inflammatory factors after an SCI is an expected observation, since after any type of injury the inflammatory cascade is activated which leads to increase concentrations of anti-inflammatory (IL-10, IL-6 and IL-9) and decrease inflammatory (IL-1β, IL-5 and IL-17) cytokines involved in the process. Previous studies have shown that there is a substantial immunosuppression after SCI (29, 41). Part of this inhibitory function seems to be related to the level of IL-10 (40). The increased level of IL-10 may reduce neuronal apoptosis and decrease caspase activity. These anti-inflammatory effects of IL-10 partially inhibit the secondary damage following SCI (42). This is an endogenous protective mechanism.

Knowledge about the prognosis of SCI patients is one of the most challenging topics in the management of these patients, since clinical examinations performed in the first few days after an insult cannot correctly determine the severity of the injury. Serum biomarkers could potentially provide a better perception of the injury's severity for the medical team. The findings of this systematic review indicated that the levels of inflammatory proteins such as IL-6, IL-8, CCL-2, CCL-4, MCP-1, TNF-α, MMP-8 and structural proteins including GFAP, S100-β and Tau are significantly lower in patients with neurological improvements during the treatment period, compared to subjects with no changes in neurological functions. This

observation also seems logical, since lower levels of these biomarkers represent a milder injury to the spinal cord, and such cases are expected to show better neurological improvements.

One of the limitations of the present review can be attributed to the fact that few of the included studies used rigorous statistical methods to assess the data. For example, only 5 of the studies reported diagnostic/prognostic accuracy of the serum and CSF biomarkers for the assessment of the severity of SCI at baseline and prognosis of neurological outcome (7, 32, 35, 36, 43). The rest of the studies only compared the mean concentration of the biomarkers between the two groups, a method that is associated with certain limitations in evaluating diagnostic value. For example, Heller et al. state that among their evaluated biomarkers only CCL-2 is able to predict AIS conversion, while the mean levels of the other serum and CSF factors they assessed also showed significant differences between the two groups of cases with and without AIS conversion (32). Therefore, a significant difference in the mean concentration of a biomarker between the two groups might not directly translate to that biomarker being an appropriate prognostic factor. The quality assessment of the included studies showed that most of them had a high risk of bias in their sample selection. The quality status for index text was also not determined in 64.7% of the studies. Moreover, the majority of the included studies were case-control and cohort studies or had performed their recruitment through a convenience sampling method. Accordingly, the findings reported by these articles have a low level of evidence.

The main problem with biomarkers is that their circulating/CSF concentration have an association with the amount of parenchyma that is affected following injury. Thus, it was better to evaluate the biomarkers association to injury severity. At the first, the authors decided to report the findings based on the severity of the injury. However, with a closer look to the included studies, it was found that only six studies (two studies in diagnostic and two in predictive values and two in both) reported the findings according to severity of SCI (27, 34, 36-39). In addition, the categorizing of the patients based on the severity of injury had considerable diversity among the studies. Therefore, it is not possible to report the results based of severity of injury. Finally, concomitant traumatic brain injury (TBI) and SCI could be falsely alter serum/CSF levels of biomarkers. Eligibility of concomitant TBI in four studies was unclear (28, 31, 34, 37) while other 12 studies excluded the TBI patients. Therefore, it seems that the prevalence of concomitant of brain injury in included subjects were low.

1.5 Conclusion

The findings of this review indicate that changes in the serum and CSF levels of inflammatory factors and structural proteins occur in response to SCI. Therefore, inflammatory factors and structural proteins can be potentially used as biomarkers for detection of SCI and can predict the subsequent neurological improvement. Although the findings of the included studies suggest that inflammatory and structural proteins may be useful in assessing the severity of SCI and prediction of neurological outcome, the level of evidence is generally low. Given limitations to the available evidence, further investigation in this field is required using large prospective datasets with rigorous analysis of sensitivity, specificity and prediction.

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1.8 Conflict of interest

The authors declare that there is no conflict of interests.

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Figure Captions

Figure 1: PRISMA flow diagram of present systematic review. The systematic search yielded 1072 non-repetitive articles. Finally, 16 studies were included.

Figure 2: Quality assessment of included studies based on Quality Assessment of Diagnostic Accuracy Studies version 2.0 (QUADAS-2) guideline.

Table 1: Search query for Medline (via Ovid)

Search terms
1. Spinal Cord Injuries/ OR Quadriplegia/ OR Paraplegia/ OR (Spinal Cord/ AND "Wounds and Injuries"/) OR (("Spinal Cord" adj (Injur* OR Contus* OR Trauma* OR Posttrauma* OR Transect* OR Lacerat* OR Compromi* OR Lesion* OR Rupture*)) OR Quadriplegi* OR Paraplegi* OR Tetraplegi* OR Quadripares?s OR ((Trauma* OR Posttrauma*) adj Myelopath*)).ti,ab.
2. Biomarkers/ OR Glial Fibrillary Acidic Protein/ OR S100 Calcium-Binding Protein A4/ OR S100 Proteins/ OR Intermediate Filaments/ OR Phosphopyruvate Hydratase/ OR Neurofilament Proteins/ OR S100 Calcium Binding Protein beta Subunit/ OR (Biomarker* OR Bioindicator* OR (Biologic* adj Indicator*) OR ((Biochemical OR Biologic* OR Clinical OR Immun* OR Laboratory OR Serum OR Surrogate Viral) adj Marker*) OR "Surrogate Endpoint*" OR "Surrogate End Point*" OR Astroprotein OR "Glial Fibrillary Acidic Protein" OR "Glial Intermediate Filament Protein" OR "Glial Fibrillary Acid Protein" OR "GFA Protein" OR "G F Protein" OR "GF Protein" OR "Glia Fibril Acidic Protein" OR "Glia Fibrillary Acid Protein" OR "Glia Fibrillary Acidic Protein" OR "Glia Filament Protein" OR "Glial Acidic Fibrillary Protein" OR "Glial Filament Protein" OR "Protein GF" OR "Protein GFA" OR "Metastasin" OR "Placental Calcium Binding Protein" OR "Calvasculin Protein" OR "Fibroblast Specific Protein 1" OR "S100A4" OR "FSP 1" OR FSP1 OR MTS1 OR "S 100A4" OR "Phosphopyruvate Hydratase" OR "2 Phospho D Glycerate Hydrolase" OR "2 Phospho D Glycerate Hydro Lyase" OR "2 Phosphoglycerate Dehydratase" OR "Phosphopyruvic Hydratase"OR "Phospho D Glycerate Hydrolyase" OR "E.C. 4.2.1.11" OR "EC 4.2.1.11" OR Enolase OR "Intermediate Filament*" OR "Intermediate Size Filament*" OR Neurofilament* OR Tonofilament* OR Calvasculin OR "S 100" OR "S100" OR "S 100beta" OR "S 100b" OR "S100beta" OR "S100B").ti,ab.
3. 1 AND 2
4. Exp Animals/ NOT Humans.sh.
5. 3 NOT 4

Table 2: Summary of included studies that assessed the diagnostic value of various biomarkers in detection of spinal cord injury

Author; Year; Country	Design	Non-SCI ; SCI	Control definition	Age	Male	Severity (AIS)	Injury level	Follow up duration (day)	Sample location	Time to sample (day)	Storage	Time storage	Biomarkers
A) Inflammatory biomarkers													
1- Interleukins													
Bank, 2015; USA	CCS	18 ; 14	Healthy	19 to 91	32	A to D	All level	15	Serum	0 to 15	NR	NR	IL-6; IL-9; IL-16; IL-18
Zaaqoq, 2014; USA	RCS	21 ; 21	Non-SCI patients	37+3	32	A to D	All level	14	Serum	1 to 14	NR	NR	IL-1; IL-5; IL-6; IL-10; IL-17
2- Chemokines													
Bank, 2015; USA	CCS	18 ; 14	Healthy	19 to 91	32	A to D	All level	15	Serum	0 to 15	NR	NR	CXCL-1; CCL-4
Hassanshahi, 2013; Iran	CCS	100 ; 78	Healthy and non-SCI patients	33.3 ± 1.6	NR	A to D	All level	90	Serum	0 to 90	NR	NR	CXCL-1; CXCL-9; CXCL-10; CXCL-12
Zaaqoq, 2014; USA	RCS	21 ; 21	Non-SCI patients	37+3	32	A to D	All level	14	Serum	1 to 14	NR	NR	MCP-1; IP-10; CCL-4
3- Interferons													
Zaaqoq, 2014; USA	RCS	21 ; 21	Non-SCI patients	37+3	32	A to D	All level	14	Serum	1 to 14	NR	NR	INF-γ
4- Other Cytokines													
Bank, 2015; USA	CCS	18 ; 14	Healthy	19 to 91	32	A to D	All level	15	Serum	0 to 15	NR	NR	MIF
B) Structural biomarkers													
1- MMPs													
Moghaddam, 2017; Germany	RCS	10 ; 20	NA	43 to 88	21	A to C	All level	90	Serum	0 to 90	-80	NA	MMP-2; MMP-8; MMP-9
2- Neurofilaments													
Ahadi; 2015, Iran	CCS	9 ; 26	Healthy	16 to 64	30	A to D	All level	3	Serum	1 to 3	-80	NR	NF-H
Kuhle, 2014; UK	CS	67 ; 10	Healthy	22-62	49	C to D	All level	7	Serum	0	NA	NA	NF-L
Ungureanu, 2014; Romania	CCS	6 ; 15	Healthy	21-59	NR	A to D	Thoracic-	24	CSF	0 to 1	NR	NR	NF-H

							cervical						
3- GFAP													
Ahadi; 2015; Iran	CCS	9 ; 26	Healthy	16 to 64	30	A to D	All level	3	Serum	1 to 3	-80	NR	GFAP
4- NSE													
Ahadi; 2015; Iran	CCS	9 ; 26	Healthy	16 to 64	30	A to D	All level	3	Serum	1 to 3	-80	NR	NSE
Wolf; 2014; Austria	CS	22 ; 12	NA	16-94	20	A to D	All level	18	Serum	1	NR	NR	NSE
5- S100-β													
Wolf; 2014; Austria	PCS	22 ; 12	NA	16-94	20	A to D	All level	18	Serum	1	NR	NR	S100-B
6- HMGB1													
Papatheodorou; 2017; USA	CCS	51 ; 11	Healthy	19-89	63	A to D	Thoracic-cervical	7	Serum	0 to 7	NR	NR	HMGB1
C) Other biomarkers													
Hosaka; 2008; Japan	CCS	36 ; 25	Healty	30-85	NR	C to D	Cervical	510	CSF	0 to 30	NR	NR	NOx
Bank, 2015; USA	CCS	18 ; 14	Healthy	19 to 91	32	A to D	All level	15	Serum	0 to 15	NR	NR	HGF; SCGF-β

AIS: American Spinal Injury Association Impairment Scale; CCL: chemokine (C-C motif) ligand; CCS: Case-control study; Cross: Cross sectional; CS: Cohort study; CSF: Cerebrospinal fluid; CXCL: Chemokine (C-X-C motif) ligand; GFAP: Glial fibrillary acidic protein; HGF: Hepatocyte growth factor; HMGB1: High mobility group box 1 protein; IL: Interleukin; INF-γ: Interferons-γ; IP-10: Inducible protein-10; MCP: Monocyte chemotactic protein; MIF: migration inhibitory factor; MMP: Matrix metalloproteinase; NA: Not applicable; NFH: Neurofilament heavy chain; NF-L: Neurofilament light chain; NOx: Nitric oxide; NR: Not reported; NSE: Neuron specific enolase; RCS: Retrospective cohort study; SCGF-β: Stem cell growth factor beta; SCI: Spinal cord injury

Table 3: Summary of included studies that assessed the prognostic value of various biomarkers in prediction of neurological improvement

Author; Year; Country	Design	Non-SCI ; SCI	Control definition	Age	Male	Severity (AIS)	Injury level	Follow up duration (day)	Sample location	Time to sample	Storage	Time storage	Biomarker
A) Inflammatory biomarkers													
1- Interleukins													
Biglari; 2015; Germany	Cross	7; 16	NA	18 to 86	16	A to D	All level	84	Serum	0 to 90	-80	NR	IL-1β
Kwon; 2017; Canada	CS	22; 26	NA	41.9 – 14.9	39	A-C	All level	180	CSF	1	NA	NA	IL-6; IL-8
2- Chemokines													
Heller; 2017; Germany	CCS	30	Healthy	10; 10	21	A to E	All level	84	Serum	0 to 90	-80	NR	CCL-2; CCL-3; CCL-4; CXCL-5
Kwon; 2017; Canada	CS	22; 26	NA	41.9 – 14.9	39	A to C	All level	180	CSF	1	NA	NA	MCP-1
3- Other cytokines													
Biglari; 2015; Germany	Cross	7; 16	NA	18 to 86	16	A to D	All level	84	Serum	0 to 90	-80	NR	TNF-α
Moghaddam; 2016; Germany	CS	26; 19	NA	42.36 ± 19.07	35	A to C	All level	90	Serum	1	-80	NA	IGF-1
Ferbert; 2017; Germany	Cross	9; 14	NA	18 to 86	16	A to D	All level	84	Serum	0 to 90	-80	NR	sCD95L; IGF-1; TGF-β1
B) Structural biomarkers													
1- MMPs													
Moghaddam; 2017; Germany	RCS	10; 10	Na	43 to 88	21	A to C	All level	90	Serum	0 to 90	-80	NA	MMP-2; MMP-8; MMP-9
2- Neurofilaments													
Pouw; 2014; Canada	CS	8; 8	NA	18-84	10	A to D	Thoracic-cervical	12	CSF	1	-80	NR	NFH
Kuhle, 2014; UK	CS	67 ; 10	Healthy	22-62	49	C to D	All level	7	Serum	0	NA	NA	NF-L
Ungureanu; 2014;	CCS	11; 4	Healthy	21-59	NR	A to D	Thoracic-cervical	24	CSF	0 to 1	NR	NR	NFH

Romania													
3- GFAP													
Kwon; 2017; Canada	CS	22; 26	NA	41.9 – 14.9	39	A to C	All level	180	CSF	1	NA	NA	GFAP
Pouw; 2014; Canada	CS	8; 8	NA	18-84	10	A to D	Thoracic-cervical	12	CSF	1	-80	NR	GFAP
4- NSE													
Pouw; 2014; Canada	CS	8; 8	NA	18-84	10	A to D	Thoracic-cervical	12	CSF	1	-80	NR	NSE
5- S100-β													
Kwon; 2017; Canada	CS	22; 26	NA	41.9 – 14.9	39	A to C	All level	180	CSF	1	NA	NA	S100-B
Pouw; 2014; Canada	CS	8; 8	NA	18-84	10	A to D	Thoracic-cervical	12	CSF	1	-80	NR	S100-B
6- Tau													
Kwon; 2017; Canada	CS	22; 26	NA	41.9 – 14.9	39	A to C	All level	180	CSF	1	NA	NA	Tau
Pouw; 2014; Canada	CS	8; 8	NA	18-84	10	A to D	Thoracic-cervical	12	CSF	1	-80	NR	Tau

AIS: American Spinal Injury Association Impairment Scale; CCL: chemokine (C-C motif) ligand; CCS: Case-control study; Cross: Cross sectional; CS: Cohort study; CSF: Cerebrospinal fluid; CXCL: Chemokine (C-X-C motif) ligand; GFAP: Glial fibrillary acidic protein; IGF-1: Insulin-like growth factor 1; IL: Interleukin; MCP: Monocyte chemotactic protein; MMP: Matrix metalloproteinase; NA: Not applicable; NFH: Neurofilament heavy chain; NF-L: Neurofilament light chain; NR: Not reported; NSE: Neuron specific enolase; RCS: Retrospective cohort study; sCD95L: Serum cluster of differentiation 95 ligand; SCI: Spinal cord injury; TGF- β1: Tumour growth factor β1; TNF-α: Tumour necrosis factor-α

Table 4: Risk of bias and applicability of included studies based on QUADAS-2 guideline.

Author, year	Risk of bias				Applicability		
	Patient selection	Index test	Reference standard	Flow and Timing	Patient selection	Index test	Reference standard
Ahadi, 2015	☹	**?**	☺	☹	☺	☺	☺
Bank, 2015	☹	?	☺	☹	☺	☺	☺
Biglari, 2015	**?**	?	☺	☺	☺	☺	☺
Ferbert, 2016	**?**	☺	☺	☺	☺	☺	☺
Hassanshahi, 2013	☹	**?**	**?**	☺	☺	☺	☺
Heller, 2017	☹	**?**	☺	☺	☺	☺	☺
Hosaka, 2008	☹	**?**	☺	☺	☺	☺	☺
Kuhle, 2014	☹	☺	☺	☺	☺	☺	☺
Kwon, 2017	☺	☺	☺	☺	☺	☺	☺
Moghaddam, 2016	☺	☺	☺	☺	☺	☺	☺
Moghaddam, 2017	☹	☺	☺	☺	☹	☺	☺
Papatheodorou, 2017	☹	**?**	☺	☹	☺	☺	☺
Pouw, 2014	☺	**?**	☺	☹	☺	☺	☺
Ungureanu, 2014	☹	**?**	☺	☹	☺	☺	☺
Wolf, 2014	**?**	**?**	☺	**?**	☺	☺	☺
Zaaqoq, 2014	☹	**?**	☺	**?**	☹	☺	☺

☺: Low risk; ☹: High risk; ?: Unclear

Table 5: Serum and CSF level of various biomarker in spinal cord injured patients compered to non-SCI subjects (diagnostic value)

Biomarkers	Time after SCI (day)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	28	90
Serum level																
IL-1β																
Zaaqoq; 2014	⇩	no	no	⇩	no	no	⇩	no	⇩	no	no	no	⇩	⇩	---	---
IL-5																
Zaaqoq; 2014	⇩	⇩	⇩	⇩	no	no	⇩	no	no	no	no	no	no	⇩	---	---
IL-6																
Bank; 2015	---	---	⇧	---	---	---	⇧	---	---	---	no	---	---	⇧	---	---
Zaaqoq; 2014	no	no	no	no	no	no	no	no	no	no	no	no	no	no	---	---
IL-9																
Bank; 2015	---	---	⇧	---	---	---	no	---	---	---	no	---	---	no	---	---
IL-10																
Zaaqoq; 2014	⇧	⇧	⇧	⇧	⇧	⇧	⇧	no	⇧	no	no	no	no	no	---	---
IL-16																
Bank; 2015	---	---	⇧	---	---	---	⇧	---	---	---	⇧	---	---	⇧	---	---
IL-17																
Zaaqoq; 2014	⇩	⇩	⇩	⇩	⇩	⇩	⇩	no	⇩	no	no	no	no	⇩	---	---
IL-18																
Bank; 2015	---	---	⇧	---	---	---	⇧	---	---	---	⇧	---	---	⇧	---	---
CXCL-1 (GRO-α)																
Bank; 2015	---	---	⇧	---	---	---	⇧	---	---	---	⇧	---	---	⇧	---	---
Hassanshahi; 2013	no	---	---	---	---	---	⇧	---	---	---	---	---	---	---	no	no
CXCL-9																
Hassanshahi	no	---	---	---	---	---	⇧	---	---	---	---	---	---	---	no	no
CXCL-10																
Hassanshahi; 2013	⇧	---	---	---	---	---	⇧	---	---	---	---	---	---	---	no	no
CXCL-12																
Hassanshahi; 2013	⇧	---	---	---	---	---	⇧	---	---	---	---	---	---	---	⇧	no
CCL-4 (MIP-1β)																
Bank; 2015	---	---	⇧	---	---	---	⇧	---	---	---	no	---	---	no	---	---
Zaaqoq; 2014	⇩	no	no	⇩	⇩	no	no	⇩	no	no	no	no	⇩	⇩	---	---
MCP-1																
Zaaqoq; 2014	no	no	no	no	no	no	no	no	no	no	no	no	no	no	---	---
IP-10																
Zaaqoq; 2014	no	no	no	no	no	no	no	no	no	no	no	no	no	no	---	---
MIF																
Bank; 2015	---	---	⇧	---	---	---	⇧	---	---	---	no	---	---	no	---	---
INF-γ																
Zaaqoq; 2014	⇩	no	⇩	⇩	⇩	no	no	no	⇩	no	no	no	no	no	---	---
MMP-2																
Moghaddam; 2017	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
MMP-8																
Moghaddam; 2017	⇧	⇧	no	no	no	no	no	no	no	no	no	no	no	no	no	no
MMP-9																
Moghaddam; 2017	no	no	no	no	no	no	no	no	no	no	no	no	no	no	⇧	no
GFAP																

Ahadi; 2015	⇧	⇧	⇧	---	---	---	---	---	---	---	---	---	---	---	---	---
Neurofilament																
Ahadi; 2015	⇧	⇧	**no**	---	---	---	---	---	---	---	---	---	---	---	---	---
Kuhle; 2014	⇧	⇧	⇧	⇧	⇧	⇧	⇧	⇧	---	---	---	---	---	---	---	---
Ungureanu; 2014	⇧	⇧	⇧	---	---	---	---	---	---	---	---	---	---	---	---	---
NSE																
Ahadi; 2015	⇧	⇧	**no**	---	---	---	---	---	---	---	---	---	---	---	---	---
Wolf; 2014	**no**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
S100-β																
Wolf; 2014	⇧	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
HMGB1																
Papatheodorou; 2017	---	---	⇧	---	---	---	⇧	---	---	---	---	---	---	---	---	---
CSF level																
Neurofilament																
Ungureanu; 2014	⇧	⇧	⇧	---	---	---	---	---	---	---	---	---	---	---	---	---
NOx																
Hosaka; 2008	---	---	---	---	**no**	---	---	---	---	---	---	---	---	---	---	---

no: No significant difference; ⇧: Significantly higher; ⇩: Significantly lower.

CCL: chemokine (C-C motif) ligand; CXCL: Chemokine (C-X-C motif) ligand; GFAP: Glial fibrillary acidic protein; HGF: Hepatocyte growth factor; HMGB1: High mobility group box 1 protein; IL: Interleukin; INF-γ: Interferons-γ; IP-10: Inducible protein-10; MCP: Monocyte chemotactic protein; MIF: migration inhibitory factor; MMP: Matrix metalloproteinase; NFH: Neurofilament heavy chain; NF-L: Neurofilament light chain; NSE: Neuron specific enolase

Table 6: Serum and CSF level of various biomarker in neurologically non-improved spinal cord injured patients compered to neurologically improved patients (prognostic value)

Biomarkers	Time after SCI (day)																	
	0*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	28	56	90
Serum Level																		
IL-1																		
Biglari; 2015	no	no	---	no	---	---	---	no	---	---	---	---	---	---	no	no	no	no
CCL-2																		
Heller; 2017	⇧	no	---	no	---	---	---	no	---	---	---	---	---	---	no	no	no	no
CCL-3																		
Heller; 2017	no	no	---	No	---	---	---	no	---	---	---	---	---	---	no	no	no	no
CCL-4 (MIP-1β)																		
Heller; 2017	⇧	no	---	no	---	---	---	no	---	---	---	---	---	---	no	no	⇧	no
CXCL-5																		
Heller; 2017	no	no	---	no	---	---	---	no	---	---	---	---	---	---	no	no	no	no
TNF-α																		
Biglari; 2015	⇧	no	---	no	---	---	no	---	---	---	---	---	---	---	no	no	no	no
MMP-2																		
Moghaddam; 2017	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
MMP-8																		
Moghaddam; 2017	⇧	⇧	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
MMP-9																		
Moghaddam; 2017	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	⇧	no	no
Neurofilament																		
Kuhle; 2014	no	⇧	⇧	⇧	⇧	⇧	⇧	⇧	---	---	---	---	---	---	---	---	---	---
sCD95L																		
Ferbert; 2017	no	no	---	no	---	---	---	⇩	---	---	---	---	---	---	no	no	no	no
TGF-β1																		
Ferbert; 2017	no	no	---	no	---	---	---	no	---	---	---	---	---	---	no	no	no	no
IGF-1																		
Ferbert; 2017	no	no	---	no	---	---	---	no	---	---	---	---	---	---	⇩	no	⇩	no
Moghaddam; 2016	no	no	---	no	---	---	---	⇩	---	---	---	---	---	---	⇩	no	⇩	no
CSF level																		
IL-6																		
Kwon; 2017	---	⇧	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
IL-8																		
Kwon; 2017	---	⇧	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
MCP-1																		
Kwon; 2017	---	⇧	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Neurofilament																		
Pouw; 2014	---	no	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Ungureanu; 2014	⇧	no	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GFAP																		
Kwon; 2017	---	⇧	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Pouw; 2014	---	⇧	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
NSE																		

Pouw; 2014	---	**no**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
S100-β																			
Kwon; 2017	---	⇧	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Pouw; 2014	---	⇧	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Tau																			
Kwon; 2017	---	⇧	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Pouw; 2014	---	⇧	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

*, time interval between 0 to 12 hours.

no: No significant difference; ⇧: Significantly higher; ⇩: Significantly lower.

CCL: chemokine (C-C motif) ligand; CXCL: Chemokine (C-X-C motif) ligand; GFAP: Glial fibrillary acidic protein; IGF-1: Insulin-like growth factor 1; IL: Interleukin; MCP: Monocyte chemotactic protein; MMP: Matrix metalloproteinase; NFH: Neurofilament heavy chain; NF-L: Neurofilament light chain; NSE: Neuron specific enolase; sCD95L: Serum cluster of differentiation 95 ligand; TGF- β1: Tumour growth factor β1; TNF-α: Tumour necrosis factor-α