Title: The Developing Landscape of Diagnostic and Prognostic Biomarkers for Spinal Cord Injury in Cerebrospinal Fluid and Blood

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The authors declare no conflict of interest.

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Abstract

Study design: Review Study

Objectives: The identification of prognostic biomarkers of spinal cord injury (SCI) will help to assign spinal cord injured patients to the correct treatment and rehabilitation regimes. Further, the detection of biomarkers that might predict permanent neurological outcome would aid appropriate recruitment of patients into clinical trials. The objective of this review is to evaluate the current state-of-play in this developing research field.

Setting: Studies from multiple countries were included.

Methods: We have completed a comprehensive literature review of all studies (to our knowledge) that have investigated prognostic biomarkers in either the blood or CSF of animals and humans following SCI.

Results: Targeted and unbiased proteomic approaches have identified several putative prognostic biomarkers in CSF and blood. These proteins associate with cellular damage following SCI and include cellular components from neurons, oligodendrocytes and reactive astrocytes, i.e. neurofilament proteins, glial fibrillary acidic protein (GFAP), Tau, and S100 calcium binding protein β (S100β). Unbiased approaches have also identified microRNAs that are specific to SCI, as well as other cell damage associated proteins.

Conclusions: The discovery and validation of stable, specific, sensitive and reproducible biomarkers of SCI is a new but rapidly expanding field of research. To date, very few studies have utilised unbiased approaches aimed at the discovery of biomarkers within the CSF or blood in this field, however some targeted approaches have been successfully used. Several studies using various animal models and some with small human patient cohorts have begun to pinpoint biomarkers in the CSF and blood with putative prognostic value. An increased sample size will be required to validate these biomarkers in the heterogeneous clinical setting.

Keywords

Spinal cord injury; biomarkers; prognostic; cerebrospinal fluid; blood; proteomics

1. Introduction

There is now a vast and expanding body of literature describing different novel approaches for the treatment of spinal cord injury (SCI). Despite this, actions to treat and rehabilitate following SCI have not changed. Outside of clinical trials, SCI is typically managed either by surgical stabilisation or conservative management in the acute and subacute setting, followed by physiotherapy in the subacute and chronic phases of injury (1,2). It is clear that the SCI research field as a whole is experiencing a significant delay in the translation of new interventions into the clinic. There are many valid reasons why scientists and clinicians alike are cautious to translate new therapies into humans, particularly as setting up appropriate clinical trials to demonstrate safety and efficacy can be difficult (3).

There is a growing appreciation for the benefit of using biomarkers to help introduce new treatments and improve strategies of care for SCI patients. We suggest there are several ways (diagnostic, prognostic and therapeutic) in which measuring biomarkers in the blood or CSF might complement current clinical measures, such as the American Spinal Injuries Association (ASIA) International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) scoring system and assessment of dry biomarkers such as magnetic resonance imaging (MRI) scans, to further the SCI field. Together a panel of biomarkers and neurological tests perhaps even including electrophysiological assessments may provide clinicians with a much clearer picture as to an individuals' severity of neurologic impairment.

Immediately following a SCI, besides those that have a complete AIS-A (equivalent to Frankel A) diagnosis where recovery of motor function, although possible (4), is relatively limited and predictable, the prognosis in the acute stage of SCI remains uncertain (5). For these patients,

knowing whether they will regain the ability to walk, irrespective of neurological, bladder or bowl function improvement, remains their key concern (6). Identification of a panel of biomarkers that could accurately predict an individuals' ability to regain neurological, physical and autonomic function, could be of great psychological benefit to these patients. Furthermore, depending on the individuals' prognosis, the treatment pathway could be tailored to ensure that optimal neurological and/or physical function is regained and that patient rehabilitative care is maintained until their best possible outcome is achieved.

ISNCSCI diagnosis of a SCI can be delayed due to problems associated with poly-trauma stabilisation or a lack of SCI expertise at the treating hospital. Therefore a diagnostic CSF or blood test that can be used to assess the neurological state of these individuals may provide a quicker, cheaper and more accurate method, which will empower clinicians to stratify patients to the most suitable treatments for their needs. Additionally, as novel treatments to target the acute phase of SCI develop, quick and accurate diagnoses of patients who will be appropriate to recruit to these clinical trials will ensure studies are appropriately powered to assess efficacy. Despite prediction of neurological improvement having been the focus of a majority of biomarker studies, there is also value in the use of biomarkers to predict other long-term outcomes, such as neuropathic pain, for which early intervention studies could be implemented to try and prevent the onset of these conditions.

Currently, in both routine clinical care and in clinical trials, the neurological condition of individuals is assessed by ISNCSCI grading and imaging modalities. Biomarkers that can easily be repeatedly measured within the blood or CSF of these individuals' to determine progressive neurological condition would be highly beneficial, as it would allow rapid determination as to whether the patient was improving, worsening or showed sustained neurological stability in response to their current

treatment; thus providing a biological surrogate outcome measure. Further, such biomarkers might indicate whether the patient has increased neurological plasticity in response to a treatment or rehabilitation regime. Finally, biomarkers released into the CSF and or blood, may provide a plethora of information as to the patients' biological response to SCI. As discussed below, different biological responses to SCI may lead to specific molecules being released into the CSF or blood; these fluids may contain a unique fingerprint that can be used by scientists and clinicians to elucidate the mechanisms underlying an individuals' SCI. Again, this could allow for personalised treatments to be provided to a patient that target their specific injury mechanisms and that can be used to assess their specific mechanistic responses.

In recent years, scientists have started to take up the challenge of discovering and validating biomarkers in the blood and CSF that have prognostic value in accurately diagnosing complete or incomplete SCI and determining SCI progression. This review aims to present an overview of the current state of play in this emerging field. We will explain how the biological process of SCI may lead to the release of biomarkers of interest into the CSF and blood; the techniques that are commonly used to find and validate these markers, and the pre-clinical and clinical studies that have already begun to highlight biomarkers of interest.

2. SCI and the release of biochemical biomarkers

This section of the review aims to highlight some of the major processes that occur following a SCI, which could lead to biomarker release. It is still unclear how biomarkers from the spinal cord are released into the blood following injury; however, we suggest that their release is likely to be highly influenced by the specific type of injury sustained and the biochemical properties of the biomarkers in question. The majority of biomarkers which have already been studied in both pre-clinical and clinical studies have been identified from targeted biomarker identification processes, i.e. looking for

markers that are likely released based on the known biological processes/mechanisms that occur following SCI.

2.1. Spinal cord tissue damage

In both animal models of SCI and in the human situation, spinal cord traumas fall broadly into two categories: transection injuries, where the spinal cord is penetrated with a sharp force; and the more common contusion traumas, where the spinal cord is essentially crushed (7,8). Both types of injury result in a breach of the blood brain barrier (BBB) and either immediate primary or secondary damage to the neurons and glia of the spinal cord tracts. Rupture of these cell types results in the release of biomarkers, largely cellular components, which are specific in the indication of nervous tissue damage and include neurofilaments (NF) (9), Tau (10), neuron specific enolase (NSE) (11), S100 calcium-binding protein β (S100β) (11) and glial fibrillary acidic protein (GFAP) (9). These tissue specific biomarkers (discussed in greater detail below) hold great promise as they are typically released into the CSF then taken up into the blood stream, allowing for their detection local to the injury site and systemically. The quantity of these proteins in the CSF and blood might directly relate to the extent of neuronal or glial damage that has occurred following SCI (12,13).

2.2 Inflammation

In brief, the breakdown of the BBB allows for an influx of inflammatory cells into spinal cord tissues. Infiltrating leukocytes and resident microglia release proteolytic and oxidative enzymes, reactive oxygen species and an array of pro-inflammatory cytokines, including, for example, tumour necrosis factor-alpha (TNF-α) (14,15). This spike in acute phase pro-inflammatory molecules can be measured in human blood in the first 24h following injury (16). Caution must be taken when considering the blood at this stage however, as many of the abundant proteins that are seen acutely after injury may be a result of the systemic response to trauma and not SCI *per se*; study of animal

models where matched ‘sham’ injuries can be performed allows for the opportunity to establish which proteins are SCI specific. The pronounced acute pro-inflammatory response to injury induces a reactive process of secondary damage in the tissues that surround the original injury site, exacerbating neuronal damage and neurological dysfunction (14). This secondary damage cascade can continue for several weeks following SCI, contributing to an expanding matrix of proteins associated with neuronal and glial cell apoptosis, such as soluble CD95 ligand (sCD95L), an initiator of the Fas apoptotic pathway (17).

2.3 Glial scarring

Glial cell activation and hypertrophy leads to the formation of a glial scar in the subacute and chronic phases of SCI (18). Astrocytes become reactive and synthesise an extracellular matrix which is effective in restoring the BBB, but that coincidentally inhibits axonal regrowth (18). The most potent of these astrocyte associated nerve inhibitory molecules are the neural chondroitin sulphated proteoglycans (CSPGs) (19,20). Myelin damage associated molecules represent the other major nerve inhibitory molecules within the glial scar, these include myelin-associated glycoprotein (MAG), Nogo-A and oligodendrocyte-myelin glycoprotein (OMgp) (21). There is a vast body of literature which confirms that CSPGs, MAG, Nogo-A and OMgp can inhibit neurite outgrowth *in vitro* and axonal regrowth *in vivo* (22–28) and that treatments which specifically target these molecules promote functional recovery in SCI pre-clinical studies both individually (29,30) and in combination (31). However, there is little research exploring the utility of these molecules as prognostic biomarkers detectable in the CSF (32). Perhaps this is because we associate such molecules with the subacute or chronic phases of injury, when a stable neurology is much more likely. However, biomarkers, such as CPSGs that could be used to monitor any transition from the sub-acute to chronic phase of injury might aid clinicians in decisions regarding rehabilitation.

3. Detection of biomarkers for SCI using unbiased approaches

Although it would be ideal, biomarkers of injury or disease are rarely either "detectable" or "undetectable". In most cases, biomarkers vary in expression levels under different conditions. It is important, therefore, to have specific and sensitive methods to quantify these changes. Typically, immunoassays have been the method of choice for studies that aimed to evaluate SCI biomarkers within the blood or CSF. The enzyme-linked immunosorbent assay (ELISA) is the most commonly employed assay to date, and both homemade and commercial ELISA kits have been utilised. Automated immunoassay systems are available for some potential biomarkers e.g. the Liaison automatic analyser for S100β and NSE (9,33), but it seems unlikely that the use of automated systems will become widespread until such biomarkers have become fully validated for routine clinical use.

The vast majority of studies aimed at finding new biomarkers for SCI have been based on a hypothesis about a particular protein of interest. Shaw et al. (2005), for example, proposed that, due to their high abundance in neurons, detection of NF proteins in CSF and/or serum is highly likely to indicate neuronal damage (34). Of the three NF subunits (i.e. light (L), medium (M) and heavy (H)), phosphorylated NF-H (pNF-H) was thought likely to be the most readily detectable in serum or CSF following neurological injury because of its relative resistance to protease degradation (34). The results from this hypothesis-driven study formed the basis of several further studies to evaluate the prognostic potential of this biomarker following SCI (9,35).

Surprisingly very few studies, however, have employed higher-throughput techniques to identify new biomarkers of SCI. A search of PubMed using the terms "proteomics AND spinal cord injury" and "biomarkers AND spinal cord injury" identified just four publications in which the aim of the study was to identify new peripherally accessible biomarkers of SCI (Table 1). Even more surprisingly,

given the popularity in other fields of biomedical research (recently reviewed by Crutchfield et al. (2016) (36)), only two of these studies reported the use of unbiased quantitative proteomic techniques to find novel biomarkers of SCI in the CSF or blood, while the remaining two studies employed relatively low-throughput array technology. Notwithstanding the limitations of array technology-based screening, several potential SCI biomarkers were identified in this way. Using a 34-cytokine sandwich ELISA microarray, Light et al. (2012), identified increased levels of matrix metalloproteinase-8 protein in CSF samples taken from adult rats at 12 days post-SCI (37), and Hachisuka et al. (2014) found increased serum levels of the microRNAs miR-9, miR-219 and miR-384-5 in mice at 12hrs after contusion SCI (n=8) compared to sham injury (n=8) using a low-density microarray platform (Table 1) (38).

Despite some findings using array technology based screening, as expected, the unbiased quantitative proteomic comparisons were more fruitful in terms of the numbers of potential biomarkers that were identified. Using difference gel electrophoresis (DIGE) and mass spectrometry (MS) analysis to compare CSF from patients at 1-8 days post SCI, Sengupta et al. (2014) identified eight proteins that were differentially expressed between complete and incomplete injured patients (39) (Table 1). Using a high-throughput label-free liquid chromatography-MS/MS quantitative proteomics technique, Lubienicka et al. (2011) compared CSF taken from rats at 24hrs post-SCI and identified 42 putative biomarkers; 10 of which are indicative of SCI severity (40) (Table 1). Moghieb et al. (2016) also used MS to identify biomarkers of SCI, however, their approach was not to initially look for CSF or blood biomarkers, instead they assessed protein changes within spinal cord tissue segments, of which Transferrin, Triosephosphate Isomerase 1, Cathepsin D and Phosphoprotein Enriched In Astrocytes 15 (PEA-15) were confirmed as altered in human SCI CSF (41).

Despite proteomics providing a popular platform for novel biomarker identification in many fields of study, other high-throughput techniques, such as lipidomics and metabolomics are also valuable in biomarker identification (36). As is the case with proteomics, only a limited number of published studies have utilised these approaches to elucidate biomarkers for SCI. Xu et al. (2015) demonstrated, by assessment of lipidomic analysis of polyunsaturated fatty-acid containing phosphatidylcholines within the spinal cord tissue, that spatiotemporal expression of one of these phosphatidylcholines matched with reactive microglia and astrocyte activity (42). Although not directly relevant to CSF or blood biomarkers, Xu et als' study indicates that lipidomic analysis of these fluids may clarify the role of lipid metabolism and damage of the cell membrane following SCI (42). There is also a need to further study the metabolome of CSF and/or blood of SCI patients, as this represents the end-point of all gene, transcript and protein interactions (43). Peng et al. (2014) published a comprehensive paper highlighting that metabolomic analysis of plasma from SCI rats led to identification of a panel of metabolites that could be used to selectively determine injured compared to sham injured animals, based on metabolite measurements alone (44). Analysis of these metabolites within the plasma of human SCI patients' is required to see if these findings translate to man and further similar metabolomic studies of human blood samples may also pinpoint other biomarkers.

4. Identifying biomarkers in the CSF and blood of pre-clinical models and human SCI patients using 'targeted' approaches

As discussed previously, the vast majority of studies that aimed to assess CSF or blood biomarkers of SCI have done so based on 'targeted' proteins that are known to relate to the biological processes that occur following a SCI. Many of these biomarkers have so far been assessed in pre-clinical models of SCI. Pre-clinical models are highly controllable and provide the opportunity to measure differences in the concentration of a biomarker in animals with a SCI and sham-injured animals (a comparison

not possible using human subjects). These models also allow for longitudinal analyses comparable to acute, sub-acute and chronic timeframes post-SCI. It is, however, difficult to relate the phases of injury in rodent models to that of the human situation, particularly as much depends on which of the models of injury are used, and as such there is no published consensus of opinion.

Causes of human SCI are wide-ranging therefore several different animal models have been generated in an attempt to account for this diversity, although it is extremely unlikely that any animal model will ever be able to replicate the complexity of human injury. As discussed previously, the two major categories of SCI are sharp force or "stab" lesions and contusive injuries. In rodent models, contusion injuries are most commonly induced using blunt force impact devices (45), in which calibrated weights are dropped onto an impounder which is rested on the surgically exposed spinal cord (46,47). This technique allows for varying degrees of injury depending on the amount of force used. Other methods of inducing an injury include the use of an aneurysm clip or calibrated forceps to compress the cord for a set time-period (48,49). Contusion injuries are commonly used as models of incomplete injury, whereas to study complete injury, complete transection of the spinal cord is often carried out using either microscissors or a scalpel blade cutting all of the spinal cord tracts by surgical incision and under visual control using suction to visually check for a complete injury (50,51).

Both human and pre-clinical models have been utilised to identify potential biomarkers of SCI progression. Tables 2 and 3 detail all of the studies (to our knowledge) that have assessed CSF and/or blood biomarkers of SCI in pre-clinical and human models, respectively. Here we discuss the leading candidate biomarkers of SCI severity and prognosis identified thus far, based on their known relevance to the biological processes that result following SCI.

4.1 Neurofilament proteins

Neurofilament proteins (NF) are the most abundant proteins in the neuronal cytoskeleton (52). They interact with other cytoskeletal proteins to regulate axonal transport and neuronal signalling (52). The presence of extracellular NF proteins is an indication of axonal damage and NF accumulation is seen in several neurological diseases (53) including multiple sclerosis (54–56), amyotrophic lateral sclerosis (54,57) and traumatic brain injury (TBI) (58). NF proteins have long half-lives (3 weeks and 2.5 months for NF-L and pNF-H, respectively) (59,60) and pNF-H, in particular, is highly resistant to breakdown by calpain and other systemic proteases (32). These proteins, therefore, provide attractive candidate biomarkers for SCI as they are not broken down before detection would be possible. The phosphorylated form of NF-H (pNF-H) (9,34) and NF-L (57,58) are the two subunits which have been most widely considered as biomarkers for SCI and shall be discussed in more detail below.

4.1.1 Neurofilament- heavy chain (NF-H)

SCI has been shown to result in increased levels of pNF-H in the CSF and blood of humans, rats and canines (9,34,61,62), as assessed using ELISA. In rat serum for example, no pNF-H can be detected, using ELISA, in uninjured and sham injured animals, however, severe experimental SCI results in high levels of measurable pNF-H (34). A detailed study of serum pNF-H concentrations (again assessed using ELISA) in rats with contusion (n=8) and spinal hemisection (n=13) injuries resulted in biphasic pNF-H being detectable in the late acute, sub-acute and chronic phases of both injuries (34). A sharp peak in pNF-H was observed at 16h post-SCI whilst maximal serum concentrations were seen at 3 days post-SCI, returning to baseline levels at approximately 18 days (34).

Animal studies have also revealed that blood pNF-H levels can indicate disease severity and directly relate to functional outcome. Nishida et al. (2012) demonstrated that in dogs with degenerative disc disease (DDD; n=60), pNF-H levels rose incrementally with the grade of injury severity observed (62). This study also demonstrated that those animals with the highest serum pNF-H levels at

veterinary presentation post-SCI were not able to regain the ability to walk following surgery (62). Ueno et al. (2011) also demonstrated a negative correlation (r -0.78) between rat plasma pNF-H levels at 3 days post SCI and hindlimb function at 28 days post SCI (assessed using Basso, Beattie, Breshnahan (BBB) score) (61).

A small cohort of human studies also indicates that there is a correlation between pNF-H and disease state. In the CSF of SCI patients (n=15), pNF-H concentrations are higher at 6 to 48h post trauma compared to that in uninjured individuals (n=6) (35). Further, Pouw et al. (2014), found that NF-H concentrations in CSF were significantly greater in motor complete (n=9) patients compared to motor incomplete patients (n=7) (9). In a recent, slightly larger study, pNF-H levels in the serum of SCI trauma patients (n=26) were significantly greater compared to controls with spinal fracture but no spinal cord trauma (n=9) at 24h and 48h post-injury (63). These studies indicate that the measurement of pNF-H within the CSF and peripheral blood has potential as a prognostic biomarker in the acute phase of SCI.

4.1.2 Neurofilament- light chain (NF-L)

Levels of NF-L have been assessed in both the CSF and serum of SCI patients (64,65). Guez et al. (2003) found there to be increased levels of NF-L in CSF following SCI compared to uninjured and whiplash injured patients (64). This study also demonstrated that for a patient with complete injury and complete tetraparesis with no long term neurological improvement, NF-L levels were 10-fold higher than in a complete injured patient who improved to AIS-D by 15-months post-injury (64). This indicates that NF-L also may have utility as a biomarker of a patients' prognosis. In the later, larger study, NF-L correlation with SCI severity and neurological outcome was confirmed (65). NF-L concentrations were found to be higher in the motor complete (n=13) patients (70 pg/mL) and motor incomplete (n=10) patients compared to others with central cord syndrome (n=4; 6 pg/mL) and

uninjured controls (n=67; 5pg/mL). Unlike pNF-H, the potential of NF-L as a biomarker for SCI has not been strengthened by pre-clinical studies. Despite this, NF-L is shown in preliminary human studies to have potential value in the classification of patients with or without capacity for neurological improvement.

4.2 Tau

Tau proteins are microtubule stabilising proteins that are highly abundant in neurons (66–68). Like NFs, these proteins function to maintain axonal transport and neuronal transmission (69). Expression of Tau proteins within the CSF or blood of animals and humans is likely indicative of neuronal damage, as these proteins are not usually secreted (10). Although several investigations into the use of Tau as a biomarker for neurodegenerative diseases, such as conversion from mild cognitive impairment to Alzheimer's disease (70), have been described, there are fewer studies examining these proteins as putative biomarkers for SCI.

There are no publications of SCI research into Tau as a biomarker in typical laboratory animal model of SCI, however, veterinary studies looking to use Tau as a marker of SCI in dogs following IVD herniation (IVDH) suggest that an acute rise in Tau levels might indicate decreased capacity for functional recovery (71). In a study of 51 dogs, CSF was collected immediately upon admission to the veterinary hospital (71). As well as Tau levels increasing with injury severity (higher in incomplete injured compared to healthy animals and in complete compared to incomplete injured animals), the highest levels of CSF Tau protein corresponded with those dogs which took the longest time to recover function (71).

In human studies, the consequence of SCI on Tau levels is not overly clear. Pouw et al. (2014) assessed Tau levels in CSF collected within 24h of injury in motor complete and motor incomplete

patients and found no significant differences associated with the degree of SCI (9). In contrast, two studies from Kwon et al. (2010 & 2016) found that in CSF collected from patients within 48h of injury with complete or incomplete SCI, Tau concentrations were significantly elevated in a severity-dependent manner (72,73). Interestingly, increased CSF Tau concentrations found between complete and incomplete injured individuals was observed at the 24h time-point (72,73), which in the Pouw et al (2014) study showed no significant difference. This discrepancy between the studies is probably due to a difference in patient numbers (Pouw et al. (2014), n=16; Kwon et al. (2010), n=27; Kwon et al. (2016), n=50) and possibly a difference in time between injury and start of CSF collection, as Pouw et al. (2014) started collecting CSF within 24h of injury (9), whereas Kwon et al (2010 &2016) started up to 48h after injury (72,73). In combination with other markers, Tau can predict initial AIS grade and if its' baseline measurement is low it can predict an improvement in AIS grade by 6 months post-injury (73).

Kwon et al. (2010) plotted Tau concentrations within the CSF from 8 to 120 hours following a SCI (72). Interestingly, the concentration of Tau remained higher in AIS-A patients compared to AIS-B and AIS-C graded patients through to 48h after injury however no difference in CSF concentrations of Tau existed between 48 and 120h post-injury (72). This observation highlights the dynamic nature of the biological processes that follow a SCI and the importance of assessing candidate biomarkers over time to ensure the most appropriate time is selected for measurement of differences in biomarkers.

4.3 Neuron Specific Enolase (NSE)

Neuron specific enolase (NSE) is the dimeric neuronal form of the glycolytic enzyme enolase. This enzyme is a marker of ischemic brain damage (74) and although it only has a short biologic half-life ($\leq$ 24h) (75), NSE holds promise as an acute indicator of neuronal damage.

NSE levels are elevated in the CSF, plasma (76) and serum (77) of rats in the acute phase of SCI. Further, NSE levels continue to be elevated at 24h post-injury in the serum of SCI compared to sham injured rats (77), however, assessment in CSF or plasma for time-periods greater than 24h post-SCI has not been evaluated in rodent models. Again, in humans NSE has only been assessed in the acute period post-injury (≤24h) (9,78) and measurement outside of this timeframe may be inappropriate with respect to the short half-life of this protein.

Nonetheless, NSE has been shown to have potential as an indicator of SCI severity. In rats with mild (n=20), moderate (n=20) and severe (n=20) spinal cord contusion injuries, 6h measurements of CSF and plasma showed significantly greater levels of NSE in moderately and severely injured rats (with greater NSE levels in the severely vs. moderately injured) compared to mildly injured animals (77). In humans, higher NSE concentrations were observed in the CSF of motor complete (n=9) compared to motor incomplete patients (n=7)(9). Results from Wolf et al. (2014) however, suggest that measurement of NSE in the serum of patients may be inappropriate to assess disease severity, as serum NSE concentrations within 24h of injury were no different when compared to vertebral injured patients with (n=12) or without (n=22) neurological deficit (78).

4.4 S100 calcium binding protein β (S100β)

S100β is a glial specific S100 protein that is released into blood and CSF during the acute phase of brain injury (79). S100β is involved in a diverse range of functions including calcium homeostasis, enzyme activity and metabolism, cell proliferation and differentiation (80). Measurement of S100β has potential as an acute marker of SCI, as it is significantly increased in the blood (76,77,81) and CSF (76) of rats at 6h after severe contusion injury compared to sham injury. In the human acute setting (<48h), S100β is also increased in the serum of patients with vertebral spine fractures

(mean=0.77 μg/L; n=34) compared to uninjured patients (0.14 μg/L; n=29) (78) and in the CSF of AIS-A grade patients compared to those with an AIS-B or C ISNCSCI score (73). Further, Pouw et al. (2014) showed there to be higher levels of detectable S100β in the CSF at 24h in those patients who did not show improvement in AIS score at 6 or 12 months post-injury (9). This finding is corroborated by Kwon et al. (2016), who showed decreased S100β concentrations within the CSF up to 48h after injury in SCI patients who demonstrated an improvement in AIS grade by 6 months post-injury (73). Therefore, early acute phase assessment of S100β within the CSF could provide a predictive biomarker of neurological improvement.

Assessment of serum and CSF S100β concentrations outside of the acute setting has not yet been studied. However, results from animal studies demonstrate that by 24h post-injury, S100β levels are unaltered in response to SCI (77), perhaps limiting the potential of this biomarker for clinical use to the acute setting only. In addition, S100β has been measured in conjunction with NSE in two animal studies (76,77) which indicated that co-measurement, rather than singular measurement of these markers in the acute stages of injury is a more robust prognostic indicator of SCI severity.

4.5 Glial Fibrillary Acidic Protein (GFAP)

The intermediate filament protein found in astroglia, glial fibrillary acidic protein (GFAP), is a widely acknowledged biomarker of severe brain damage resulting from haemorrhage or serious trauma, with both serum and CSF levels being higher in patients with traumatic brain injury (TBI) compared to uninjured controls (82). Despite the fact that GFAP is an established marker of neural injury in other fields, very few studies have investigated its potential as a biomarker of SCI. In a small preliminary study, Yokobori et al. (2015), demonstrated higher GFAP levels in the CSF of rats in the acute phase following contusion injury (n=4) compared to sham injured animals (n=4) (83). Ahadi et al. (2015) (63) demonstrated that GFAP is also increased in the serum of human acute SCI

patients (n=26) compared to uninjured controls (n=9). Further, Pouw et al. (2014) and Kwon et al. (2016) confirmed that CSF GFAP concentrations were higher in complete vs. incomplete SCI patients and hence that GFAP concentrations appear to be associated with SCI severity (9,73). Measurement of CSF GFAP within 48h of injury has also been used, in combination with other inflammatory and structural markers, to predict which AIS-A patients would show an improvement in AIS score by 6 months post-injury, with an 83% success rate (73). Therefore acute assessment of CSF GFAP may provide a predictive biomarker of neurological improvement. Longitudinal analyses by Yokobori et al (2015) (83) showed maximal GFAP levels in CSF in rats at 4h post SCI, with CSF concentrations decreasing sequentially at 24h and 48h after injury (83); further studies are required to ascertain GFAP levels in the chronic phase of SCI.

4.6 Pro-inflammatory cytokines

Unsurprisingly, SCI can lead to the release of pro-inflammatory cytokines across the BBB. Therefore, several researchers have investigated whether concentrations of these cytokines in the blood of SCI patients relate to neurological outcome. TNF-α is a cytokine involved in the acute phase of pro-inflammatory signalling and is increased in the serum of SCI patients (n=56) compared to uninjured controls (n=35) in the sub-acute phase (2-52 weeks) (84). This pattern of increased serum TNF-α concentrations following SCI (n=6) compared to sham injury is maintained in rats (85). Moreover, SCI patients who show improved neurological function, had lower TNF-α at 9h, compared to SCI patients who failed to improve neurologically (16). Interleukin 1 beta (IL-1β) is a key moderator of proliferation and inflammation that is thought to be vital for the formation of the glial scar (86). Ischaemia/ reperfusion SCI in rats (n=6) resulted in increased serum IL-1β levels at both 24 and 48 hrs after injury when compared to sham injured rats (n=6) (85). Despite human CSF or blood measurements of IL-1β not having been compared between SCI and uninjured individuals, baseline assessment (4 hrs after hospital admission) of this cytokine in serum showed no difference between patients who did or did not show an improvement in AIS score (16). Between weeks 1 and 4

after injury, however, serum IL-1β concentrations decreased significantly, only in patients who did not show an improvement in AIS score (16), indicating that maintenance of higher serum IL-1β concentrations may lead to improved neurological outcome. Previously, a pre-clinical model has also indicated that Interleukin 6 (IL-6) may be a suitable blood biomarker to diagnose SCI, as at both 24 and 48 hrs after SCI serum concentrations of IL-6 were greater when compared to sham injured rodents (85). More recently, Kwon et al (2016) have demonstrated CSF concentrations of pro-inflammatory cytokines IL-6 and Interleukin 8 (IL-8) can be assessed in the acute phase of human injury (≤48h) to both determine injury severity and to predict neurological improvement from an AIS-A to either AIS-B or C grade by 6 months post-injury (73).

4.7 Soluble CD95 ligand (sCD95L)

During the acute and subacute phase of SCI, neuronal damage via apoptosis is prolific. The Fas ligand receptor system is key in driving this apoptotic response (87). Soluble CD95 ligand (sCD95L/Fas-L) is a cleavage product of the type II transmembrane protein CD95L (17), which when activated and bound to CD95 (Fas) can initiate the Fas apoptotic pathway. sCD95L induces neutrophil secretion of pro-inflammatory chemokines (88). Although blocking the CD95 pathway in SCI rats improved functional outcome, assessment of human blood sCD95L via ELISA, showed no difference in concentration when comparing complete vs. incomplete injured patients at 4h and 12 weeks post injury (89,90). It is of note, however, that in these human studies no uninjured control group was included; as such it is difficult to determine whether sCD95L concentration alters at all in response to SCI.

5. Discussion

This review has aimed to evaluate biomarkers in the CSF and/or blood that are currently under assessment as potential indicators of SCI diagnosis, severity and likely neurological outcome in

preclinical and clinical studies. These studies have aimed to establish whether biomarker detection in CSF and blood is possible, to determine the longevity and stability of these biomarkers in each body fluid, and their value in predicting neurological outcome, as assessed by ISNCSCI score. All of the studies described are either in the pre-clinical stages of biomarker validation or have been undertaken only in a small number of human patients. Pre-clinical models provide an invaluable tool in which biomarker characteristics can be studied without the added complexity of clinical human-to-human SCI variability. Importantly, the use of sham-injured animals for comparison ensures that biomarkers that are specific to SCI are identified, as sham-injury can account for systemic responses, such as systemic inflammation, that may occur in relation to the 'trauma' of sham injury. In human studies that have compared biomarkers between SCI and healthy 'controls' (91), such healthy individuals are unlikely to demonstrate any of the systemic biological responses that may exist, therefore some of the protein differences observed between the injured and control groups are likely to be non-specific to SCI. Access to appropriate human 'sham injury controls', where the same level and type of trauma is observed along with matched patient demographics but without any injury to the spinal cord tissue is impossible to obtain. Guez et al. (2003), however, have assessed the utility of comparing SCI patients to individuals who had severe whiplash as a form of human 'sham' injured control. The majority of candidate biomarkers in the described literature represent neural structural proteins which are likely to be damaged following SCI and released into the CSF and blood following disruption of the BBB. A cautionary aspect to consider for these SCI biomarkers is that some are known to increase in the CSF and blood of individuals with brain injury or nervous system disease (58,74,79,82); these confounding factors should be taken into consideration when exploring their utility in the clinic, especially in incidences of polytrauma. Further, some of the biomarkers that have indicated potential in SCI biomarker development have a short half-life (e.g. NSE), therefore accurate measurement of these may need to be carried out immediately after injury. Unfortunately,

500 the assessment of SCI biomarkers in the acute setting (<24h) might not always be possible,
501 particularly in complex polytrauma cases where patient stabilisation is the priority.
502
503 Several of the studies included in this review have assessed biomarkers solely within the CSF. It is
504 intuitive to think that body fluids local to the injury site will contain the highest concentration of SCI
505 specific molecules, metabolites or proteins. This has been confirmed by studies that have directly
506 compared human biomarker concentrations in matched CSF and blood samples, which have
507 demonstrated that acutely after injury (≤48h) concentrations of IL-6, IL-8, MCP-1, Tau, S100β and
508 GFAP were at least 10 fold higher in the CSF compared to the blood (72); much higher CSF
509 concentrations of biomarkers, including GFAP, were also demonstrated by Yokobori et al. (2015)
510 (83). The collection of CSF from SCI patients however, increases their risk of infection of the
511 meninges and has cost implications for the health service provider (92). Alternatively, if biomarkers
512 can be identified systemically, the collection and analysis of peripheral blood would represent a less
513 risky and more cost-effective approach. Therefore, there is benefit in pursuing techniques that are
514 sensitive enough to detect differences in biomarker concentrations in blood, however, initial
515 assessment of potential biomarkers may best be carried out in CSF where more apparent changes are
516 likely to be noted.
517
518 The majority of published studies that have assessed blood or CSF biomarkers in human SCI patients
519 have assessed the effectiveness of a biomarker based on its ability to predict or correspond to
520 ISNCSCI score. However, it may be that other measures of progression, such as improvements in
521 hand grasping, medical imaging or electrophysiology provide more subtle improvements, which
522 could more easily be unpicked by a difference in biomarkers.
523

The use of unbiased approaches to screen for putative biomarkers of SCI progression in CSF and blood, for example quantitative proteomic approaches, have so far been largely overlooked, but are likely to yield the greatest number of novel biomarker targets. The limited proteomic analyses of CSF from SCI patients that exists provides a benchmark for the number of novel candidates that can be identified (41), however, there is currently a lack of any essential follow-on validation via quantitative western blot or ELISA. An alternative approach to identifying novel biomarkers using a high-throughput approach, may be to assess protein changes within the spinal cord tissue and then evaluate whether these changes are reflected in the CSF or bloods, as could be demonstrated by Moghieb et al. (2016) (41). Alternatively, as bioinformatic approaches aimed at interpreting large proteomic datasets improve, initial *in silico* validation of the candidate biomarkers might be possible as an interim step before completing costly quantitative validation; an approach which has been effective in Alzheimer's disease (93).

In this review, we have evaluated the current state-of-play in the CSF and/or blood biomarkers of SCI research landscape, this review highlights some of the potential pitfalls which need to be overcome to ensure the clinical utility of biomarker candidates, such as accounting for polytrauma and delayed SCI diagnoses. In addition, it is clear that further investigation is required, to include much larger cohorts of human participants with a diverse range of injuries in order to confirm the clinical validity of the preliminary biomarker findings described. The need to identify and validate novel prognostic biomarkers that can be measured within the blood or CSF, for the assessment of SCI progression using unbiased approaches has also been discussed.

It is highly unlikely that a single biomarker measurement will ever be used on its own to accurately predict SCI recovery in the clinic. We suggest that demographic and injury associated risk factors as well as the evaluation of 'dry' biomarkers i.e. radiological imaging modalities and

electrophysiological measurements in combination with the quantitation of several validated CSF and/or blood biomarkers will ultimately be used to provide a 'risk of SCI progression' index. Such a prognostic risk index would greatly advance the clinical management of SCI patients, reducing uncertainty for both patients and health care providers in the acute SCI setting and providing confidence in neurological stability prior to the recruitment of SCI patients into clinical trials.

Finally, this review highlights the fact that very few studies have been published to identify biomarkers for other uses in the SCI field. Undoubtedly, biomarkers that could be used in clinical trials that aim to target specific disease mechanisms, such as remyelination, would be invaluable for assessing efficacy of a particular treatment and the mechanism of interest. Further, biomarkers that could be used to identify patients who will develop other long-term problems, such as neuropathic pain would also be advantageous for the stratification of patients to particular treatment.

561

Reference	Injury Type	Sample numbers	Species	Sample	Time of sampling (after SCI)	Method of Biomarker screening	Candidate Biomarkers	
Light et al., 2012 (37)	Contusion Sham	n=4 n=4	Rat	CSF	12 days	Cytokine ELISA microarray	Matrix Metalloprotease-8 Thymus Chemokine-1	
Hachisuka et al., 2014 (38)	Contusion (mild) Contusion (severe) Sham Untreated	n=8 n=8 n=8 n=8	Mouse	Serum	12h	Taq-man low density array	miR-219 miR-384-5p miR-9	
Sengupta et al., 2014 (39)	Complete Incomplete Complete Incomplete	n=7 n=8 n=3 n=3	Human	CSF	1-8 days (acute) 15-60 days (sub-acute)	Difference gel electrophoresis (DIGE) and matrix assisted laser desorption/ ionisation-mass spectrometry (MALDI-MS)	GTF3C5 HP IGHG2 IGHG4	ALB TF AZGP1 APOH
Lubienicka et al., 2011 (40)	Contusion (moderate) Contusion (severe) Sham	n= 9 n= 9 n= 9	Rat	CSF	24h	Liquid chromatography-mass spectrometry (LC-MS/MS)	YWHAG ORM1 A1M A2M APOA1 APOH B2M CA1 CA2 C3 C1 CRP FAM3C GPX3 ITIH4 ITIH3 LASMP F11R KNG1	LDHA IGKC NBL1 SCG5 PRDX2 PZP ZMYND8 S100A8 F2 SCG3 SERPINC1 CDH13 MAP1 YWHAZ

Table 1 Candidate blood and/or CSF biomarkers for SCI identified from high-throughput techniques

Reference	Biomarker	Injury type	Sample numbers	Species	Sample	Time of sampling (after SCI)	Findings
Ueno et al., 2011 (61)	pNF-H	Moderate contusion	n=4	Rat	Plasma	1, 2, 3, 4 days	Investigated if minocycline treatment could improve recovery following SCI by looking at pNF-H as a potential biomarker. pNF-H was detectable from 1 day post SCI, with levels peaking at 3 days. pNF-H levels were lower in rats which had improved hindlimb function (BBB score). A negative correlation between pNF-H level at 3 days post SCI and BBB score at 28 days post injury existed.
Nishida et al., 2012 (62)	NF-H	Paraplegia with IVDH	n=60 control: n=6	Dog	Serum	1-3 days	pNF-H was higher in animals with worse paraplegia (grade 5 vs grade 4). Eight dogs with the highest pNF-H levels were unable to walk following surgery.
Shaw et al., 2005 (34)	pNF-H	Contusion Spinal hemisection	n=8 n=13	Rat	Serum	5, 2, 8, 16, 24h 2-21 days	Increased pNF-H in SCI (contusion and spinal hemisection) injured vs. sham injured. pNF-H increased in the first few hours of injury and peaked at 16h post SCI. pNF-H levels had a second high peak observed at 3 day post SCI before returning to baseline levels at 18 days post SCI.
Roerig et al., 2013 (71)	Tau	IVDH	n=51	Dog	CSF	At time of veterinary admission	Tau levels were increased in dogs with motor complete injury compared to healthy or motor incomplete injured dogs. Dogs which improved at least one neurological grade within a week had lower tau concentrations than those that took longer to recover.
Loy et al., 2005 (77)	NSE; S 00β	Moderate contusion Severe contusion	n=12 n=10	Rat	Serum	6, 24h	Significantly higher serum NSE levels were noted at 6h and 24h following SCI compared to sham injured animals. Significantly higher serum S100β levels at 6h in severely injured rats. S100β levels were not significantly different wh n comparing SCI and sham injured rats at 24h.

Cao et al., 2008 (76)	NSE; S100β	Mild contusion Moderate contusion Severe contusion	n=20 n=20 n=20	Rat	CSF; S ru m	30 mins 2,6,12,24h	Significant increase in NSE and S100β levels in both serum an CSF from 2h post SCI compared to sham injury. At 6h post SCI, CSF and plasma NSE and S100β were significantl higher in moderate and severely injured rats compared to m ldl injured rats and were significantly higher in severely injured rat compared to moderatel injured rats.
Ma et al., 2001 (81)	S100	Spinal compression	n=40 control: n=24	Rat	Serum	2, 6, 13, 24h 3, 6, 10 days	Serum S100 increased within 3h after injury in the SCI rats. Levels of serum S100 peaked at 3h, 12h and 3 days after SCI an was significantly higher than levels in serum of sham injured rats a all three time points tested.
Yokobori et al., 2015 (83)	GFAP; SBDP120; SPDP145	Contusion	n=4	Rat	CSF	4, 24, 48h	GFAP and UCH-L1 levels in the CSF were increased at 4h, 24h and 48h post SCI compared to sham injury. CSF GFAP levels were highest at 4h post injury, then decreased a 24h and 48h. UCH-L1 was increased at 4h but not 24h or 48h after SCI whe compared to sham injured animals.
Hasturk et al., 2009 (85)	TNF-α IL-1β IL-6	Spinal ischemia/ reperfusion	n=6	Rat	Serum	24, 48h	Serum TNF-α, IL-1β and IL-6 was elevated following ischemi reperfusion injury compared to sham injury at 24 and 48 hrs. None of the cytokines showed altered abundance at 24 compared t 4 hr in injured rats.
Hachisuka et al., 2014 (38)	miRNA	Mild contusion Moderate contusion	n=8 n=8	Mice	Serum	3, 12, 24h 3, 5, 7, 14, 21, 28, 35, 42 days	miR9 and miR384-5p were significantly higher in mouse serum a 3h, 12h, 24h and 72h following SCI compared to sham injured mice miR219 was significantly higher in mouse serum at 3h, 12h and 24h following SCI compared to sham injury.

Table 2 Biomarkers of SCI identified and/or validated using animal models

Abbreviations: BBB, Basso, Beattie, Breshnahan score; CSF, cerebrospinal fluid, IVDH, intervertebral disc herniation; NF-H, neurofilament heavy chain; NSE, neuron specific enolase; GFAP, glial fibrillary acidic protein; S100β, S100 calcium binding protein β; SCI, spinal cord injury

Reference	**Biomarker**	**Patient groups**	**Sample numbers**	**Spinal Level (n)**	**AIS Grade (n)**	**Age [y] Mean (*Range*) M/F ratio**	**Sample/ Assay Type**	**Time of sampling (post-injury)**	**Findings**
Ahadi et al., 2015 (63)	GFAP; pNF-H; NSE	Traumatic SCI Control (Spinal fracture, no trauma)	n=26 n=9	C (8) T (8) L (10)	A (10) B (7) C&D (9)	All (n=35) 37 (*16-64*) 30/5	Serum/ ELISA	24h; 48h; 72h	GFAP sig. increased in trauma SCI vs controls at all time points. GFAP related to SCI severity. pNF-H & NSE sig. increased in trauma SCI vs controls at 24 & 48h after injury.
Biglari et al., 2013 (89)	sCD95L	Traumatic SCI	n=8	C (5) T (3)	A (2) B (1) C (3) D (2)	48 (*18-86*) 5/3	Serum/ Immuno-assay	24h; At day 3, 7, 14, 28 & 90	No difference detected between patients, but levels decreased during the 1st week, increased during the 2nd week, were highest in the 4th week and levels plateaued at 12 weeks.
Biglari et al., 2015a (90)	sCD95L	Traumatic SCI	n=23	C (8) T (9) L (6)	A (15) B (6) C (2)	43 (*18-85*) 16/7	Serum/ Immuno-assay	On admittance; 4, 9, 12 & 24h; 3 & 7 days; 2, 4, 8 & 12 weeks post-admission	sCD95L was significantly reduced during the first 24h, but was significantly higher c.f. admission levels

									at 8 weeks.
Biglari et al, 2015b (16)	IL-1β; TNF-α	Traumatic SCI	n=23	C (8) T (9) L (6)	A (15) B (6) C (2)	43 (*18-85*) 16/7	Serum/ Immuno-assay	On admittance; 4, 9, 12 & 24h; 3 & 7 days; 2, 4, 8 & 12 weeks post-admission	Improvers were found to have lower TNF-α at 9h c.f. non-improvers. IL-1β declined in all patients between 2 & 12 weeks.
Davies et al., 2007 (84)	IL-1β, IL-6, TNF-α, IL-4, IL-10, IL-2, IL-1RA, myelin-associated glycoprotein, GM_1 ganglioside IgG (G & M)	Traumatic SCI Control	n=56 n=35	Between C4 & T12	A (14) B (13) C (22) D (7)	41 42/14 35 (*18-65*) 18/17	Serum/ ELISA	1st visit at rehab 22 (2-52 wk post-injury) 34 (>52 wk)	Excluded patients with communicable diseases, cancer diagnosis or on anti-inflammatory medication also with nontraumatic aetiologies such as epidural abscess, aneurysm etc. IL-6, TNF-α, IL-1RA & anti-GM was increased in SCI patients c.f. controls. These levels are increased further in SCI patients presenting with neuropathic pain, UTIs & pressure ulcers.
Guez et al., 2003 (64)	GFAP; NF-L	Cervical fracture dislocation with neurological deficit Severe whiplash with neurological deficit Control (no	n=6 n=17 n=24	C (6)	A (3) B (1) D (2)	48 (*40-69*) 5/1 39 (*26-56*) 11/6 31 (*23-56*) 12/12	CSF/ ELISA	1-21 days	Exclusions included patients with head injury or unconsciousness. GFAP & NF-L increased in cervical fracture dislocation group. NF-L was

		neurology)							increased in 3 patients with whiplash indicating axonal injury.
Kuhle et al., 2015 (65)	NF-L	Motor-complete SCI CCS Motor-incomplete SCI Healthy controls (no neurological Deficit)	n=13 n=4 n=10 n=67	C (11) T (2) C (4) C (9) T (1)	A (12) & B (1) C (2) & D (2) C (7) & D (3)	32 (*22-45*) 8/5 49 (*39-62*) 3/1 33 (*22-43*) 7/3 35 (*28-42*) 29/38	Serum/ In-house immuno-assay	12h & every 12h subsequently up to 7days	NF-L correlated with severity & neurological outcome.
Kwon et al., 2010 (72)	25-plex cytokine array plus IL-16 & growth factors; Tau; S100β; GFAP	Complete SCI Incomplete SCI Controls (undergoing operations for hip, knee or spine)	n =14 n=13 n=12	C (11) T (3) C (10) T (3)	A (14) B (7) & C (6)	All (n=27) 48 (*20-66*) 19/8	CSF & Serum/ ELISA & Multiplex array system	≤72h	Exclusions – concomitant head injuries, major trauma to chest, pelvis or extremities requiring intervention or if too sedated or intoxicated to assess neurology. Produced a biochemical model using a combination of S100β, GFAP & IL-8 from CSF to reliably (89% of patients) predict injury severity (AIS- A, B or C) at 24h post-injury. These markers also predicted segmental motor recovery at 6 months.

Kwon et al., (2016) (73)	Tau, S100β GFAP IL-6 IL-8 MCP-1	Traumatic SCI	n=50	C (32) L (3) T (15)	A (29) B (12) C (9)	41.9 4/1	CSF/ ELISA	≤48h	GFAP, IL-6, S100β and Tau were significantly different between AIS- A, B and C grade individuals. A discriminant function analysis model showed 83% success rate at predicting baseline AIS grade based on CSF concentrations of all of these biomarkers together. Baseline concentrations of IL-6, IL-8 MCP-1, Tau, S100β and GFAP were different between those who showed neurological improvement (conversion of AIS grade 6 months) compared to those with the same AIS grade at 6 months.
Pouw et al., 2014 (9)	GFAP; NSE; S100β; Tau; NFH	Motor-complete SCI Motor-incomplete SCI	n=9 n=7	C (6) T (3) C (5) T (2)	A (7) B (2) C (4) D (3)	All (n=16) 46 (*18-84*) 10/6	CSF/ ELISA	≤24h	Patients requiring interventions for major trauma to chest, pelvis and/or extremities or with pre-existent neurodegenerative disorders were

									excluded. NSE, S-100β & NFH were increased in motor-complete c.f. motor-incomplete patients.
Ungureanu et al., 2014 (35)	pNF-H	Complete SCI Incomplete SCI Normals	n=8 n=7 n=6	C (6) T (2) C (4) T (3)	A (8) B,C, D (7) E (6)	35 (*21-53*) 6/2 45 (*33-59*) 5/2	CSF/ ELISA	6-12h, then daily until discharge or death	Patients presenting with TBI & chronic CNS pathologies were excluded. pNF-H was detectable in all SCI patients, but was more elevated in complete SCI.
Wolf et al., 2014 (11)	NSE; S100β	Vertebral spine fractures with neurology deficit Vertebral spine fractures with no neurology deficit Control (acute fractured femur)	n=12 n=22 n=29		Complete (5) Incomplete (6) Parasthesia (1)	Spinal fracture (n=34) 53 (*16-94*) 20/14 77 (*22-94*) 8/21	Serum/ Immuno-assay	≤ 24h	Patients excluded were those with TBI, requiring intubation or unstable, open fractures, pregnancy, polytrauma or severe penetrating injuries. S100β was increased in patients with vertebral fractures and was significantly highest in patients with neurology deficit.
Yokobori et al, 2015 (83)	UCH-L1; SBDPs; MBP; GFAP	Moderate-severe SCI Non-SCI (with hydrocephalus or unruptured	n=7 n=15		A, B & C (7)		CSF & serum/ ELISA	≤ 24h	Preliminary data suggesting that the structural proteins UCH-L1 & SBDPs may be

		aneurysm)							biomarker candidates for SCI .

570

571 **Table 3 Biomarkers used in traumatic human SCI**

572 Abbreviations: CSF, cerebrospinal fluid; NF-H, neurofilament heavy chain; NSE, neuron specific enolase; GFAP, glial fibrillary acidic protein; S100β, S100
573 calcium binding protein β; SCI, spinal cord injury; TBI, traumatic brain injury.

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