Spinal Cord Injury: How Can We Improve the Classification and Quantification of Its Severity and Prognosis?

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Abstract

The preservation of functional neural tissue after spinal cord injury (SCI) is the basis for spontaneous neurological recovery. Some injured patients in the acute phase have more potential for recovery than others. This fact is problematic for the construction of clinical trials because enrollment of subjects with variable recovery potential makes it difficult to detect effects, requires large sample sizes, and risks Type II errors. In addition, the current methods to assess injury and recovery are non-quantitative and not sensitive. It is likely that therapeutic combinations will be necessary to cause substantially improved function after SCI, thus we need highly sensitive techniques to evaluate changes in motor, sensory, autonomic and other functions. We review several emerging neurophysiological techniques with high sensitivity.

Quantitative methods to evaluate residual tissue sparing after severe acute SCI have not entered widespread clinical use. This reduces the ability to correlate structural preservation with clinical outcome following SCI resulting in enrollment of subjects with varying patterns of tissue preservation and injury into clinical trials. We propose that the inclusion of additional measures of injury severity, pattern, and individual genetic characteristics may enable stratification in clinical trials to make the testing of therapeutic interventions more effective and efficient. New imaging techniques to assess tract injury and demyelination and methods to quantify tissue injury, inflammatory markers, and neuroglial biochemical changes may improve the evaluation of injury severity, and the correlation with neurological outcome, and measure the effects of treatment more robustly than is currently possible. The ability to test such a multimodality approach will require a high degree of collaboration between clinical and research centers and government research support. When the most informative of these assessments is determined, it may be possible to identify patients with substantial recovery potential, improve selection criteria and conduct more efficient clinical trials.

Key words: clinical trials; neurological recovery; neuroprotection; spinal cord injury

Introduction

THE HISTORY of experimental therapeutics clinical trials in spinal cord injury (SCI) has taught the research and clinical communities several lessons. One of the most important is that SCI is a heterogeneous condition for which oversimplified classification can preclude the ability to find an effect of an experimental therapeutic, even if it is present in some subjects. Clinical trials require substantial resources and it is important to maximize their value and control their costs. After SCI, the difference between

recoveries of substantial or minimal neurological function is determined by the preservation of only a fraction of the original tissue at the injury site.¹ In this review, we discuss how the advance of testing experimental treatments for SCI is limited by our current injury classification methods, the lack of techniques to define the structural basis of preserved neurological function, and the need for sensitive quantitative prediction and outcome measures.

The heterogeneity of SCI is due to factors that include variations in injury severity and pattern, individual genetic differences,

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pre-injury health issues, variability in medical, surgical and rehabilitative care, and individual variation in sequelae during chronic SCI. Existing SCI classification systems, although of proven value, artificially diminish this variability, limiting the correlation of individual neurological status with outcome, especially for incomplete injuries. This heterogeneity affects SCI therapeutics clinical trials because subjects with similar American Spinal Injury Association (ASIA) impairment classifications but differing in other respects are enrolled into the same group in a study. A positive effect of the heterogeneity is to increase the external validity of the study if a benefit is found.^{2,3}

A consequence of oversimplified initial classification is that it can create so much variation in the outcome data that a benefit from treatment cannot be detected, leading to a false negative conclusion (Type II error). In addition, awareness of this heterogeneity can lead to power analysis calculations of the number of subjects needed to be randomized to conduct a valid statistical analysis that are financially or logistically prohibitive. The impact of these current realities is that some potentially important trials are evaluated as being too financially risky and fail to go forward, to the detriment of the whole SCI field. Efforts are underway to find alternative acute and chronic measures to quantify injury, assess spinal cord structure and neurophysiology, and define patient-specific factors to improve prognosis, optimize treatment, define mechanisms of action, and stratify enrollment in SCI clinical trials. Advances in imaging, detection and quantification of tissue damage and inflammatory markers, elucidation of genes linked to greater or less recovery potential and determination of individual pharmacokinetic and pharmacodynamics variations of administered therapeutics should lead to more accurate descriptions of an individual SCI, enhanced prognostic accuracy, dose optimization, and improvements in the matching of persons with SCI to suitable experimental therapeutics.^{4,5}

It may become possible to combine clinical neurological assessment, imaging data, quantitative injury biomarker, and pharmacokinetic/dynamic, genetic and other information into predictive models if sufficiently large sets of patient-specific data are created from which to derive such models⁶⁻⁸ and the non-linear relationships of the variables can be accommodated.

For treatments such as cellular therapies, the input of patient-specific information to predictive algorithms and assessment with imaging technology with superior spinal cord resolution may avoid enrollment of subjects whose injuries are so severe that there is little or no prospect of benefit. This is especially important to reduce trial costs and increase efficacy potential when the experimental treatment is combined with heavy resource utilization such as extensive rehabilitation. For example, if a therapy is designed to promote remyelination, it would be important to recruit subjects in whom evidence of demyelination could be established.

The concept that one treatment will be sufficiently robust to improve the outcome of diverse SCI patients is inherently flawed and derived from extrapolations of simpler organ systems and diseases. The view that a single treatment should be sufficiently "robust" to achieve statistical significance in the primary endpoint for a heterogeneous population may be too stringent if there are a considerable proportion of non-responders. The SCI field needs to design studies that enhance the probability of detecting clinical success with therapeutics. Prior "failed" therapies may have been unsuccessful due to dilution of effect in overly heterogeneous patient groups. Examples of such include NASCIS⁸ and Sygen,⁹ where large numbers of subjects were

enrolled but not stratified with regard to lesion completeness and injury region (e.g., cervical vs. thoracic SCI). Further, it is now clear that the Sygen primary outcome of a two-grade increase in the ASIA impairment score was not an optimal outcome measure.

In addition to a need for more specific and quantitative variables to better describe an individual SCI patient, there also is a need for additional sensitive and specific outcome measures that can identify and quantify small changes in neurological function and connectivity of the spinal cord to reduce the risk of a false negative conclusion in Phase I and II neuroprotection, neuror-epair, and neuroplasticity clinical trials. Although Phase III studies require functional outcome measures and prospective definition of a minimal clinically significant change,¹⁰ early phase studies of new treatments such as drugs and biologics need sensitive detection methods to verify biological activity and detect small effects on neurological function. Without the ability to detect small effects, it is difficult to optimize variables such as dose and therapeutic window and design the combination therapies that will likely be needed to obtain larger aggregate effects leading to useful recovery.

Another important issue related to the assessment of acute SCI is that methods to detect worsening of injury are very limited during the acute phase where widespread transient axonal dysfunction and spinal shock can mask reversible neurological deficits.¹¹ Usually, the neurological deficit associated with SCI is maximal at onset because the initial injury causes severe acute dysregulation of axon conduction. Worsening of the presenting deficit (e.g., ascent of neurological level, increasing weakness of extremities, bladder failure, impaired breathing) is not expected with standard-of-care management but is diagnosed after clinically detectable and potentially irreversible deficits appear. Currently, the only practical real-time method to detect worsening injury is by repeated clinical examination, wherein deterioration evident as ascent of neurological level or loss of preserved function below the injury level may not be reversible. Because accurate clinical exams are often challenging or impossible in the early period after SCI,^{12,13} preventable occult neurological damage due to compression, occult motion, and ischemia can occur as a result of inability to assess the status of spinal cord tissue function in terms of metabolic state, tolerance to ongoing compression, and other factors. Because of this lack of ability to monitor spinal cord physiology in real time, current clinical measures emphasize best practices, such as control of systemic mean arterial pressure (MAP), spinal immobilization, and expeditious decompression.^{14,15} The Subaxial Injury Classification System was developed to classify cervical injuries and also to predict those at greatest risk of deterioration without appropriate decompression and stabilization.¹⁶ Because the clinical neurological examination in the acute phase after SCI may be unreliable,^{17,18} it is important to develop additional objective measures of the physiology, function, and viability of the spinal cord to pre-emptively detect reversible changes in the injured spinal cord tissue.

Injury completeness is a pivotal issue in SCI. Even minor detectable sensory and motor function during the acute or early recovery phase has major implications for recovery.¹⁹ The majority of experimental therapies seek to either preserve tissue or to augment repair of residual axons. If there are no viable axons crossing the injury epicenter, then many forms of therapy directed at preserved axons are futile. It would be very useful to know the threshold of spared tissue below which clinical functions cannot be detected in humans. For non-acute studies that employ

targeted interventions, such as cellular therapy, accurate measures of the anatomical extent of an SCI and its specific patterns of pathophysiology (e.g., spared axonal pathways,^{20,21} axonal demyelination,²² state of the inflammatory response and injury cavity volume and shape) could allow a more rational selection of research subjects, timing of treatment, site of therapeutic delivery, and dosing.

Current Clinical Classification of Acute Spinal Cord Injury

The physician treating SCI primarily manages a patient based on vital signs, careful and frequent neurological examination, and imaging studies, especially magnetic resonance imaging (MRI). Clinically, SCI is most fundamentally classified into complete and incomplete neurological loss based on the presence or absence of perianal sensory function.²³ Then, a determination of injury level and density is made based on motor strength in key muscles, pinprick and light touch sensory perception at selected dermatomal points in accordance with the “*International Standards for Neurological Classification of Spinal Cord Injury* (ISNCSCI).^{24–26} An ISNCSCI A is a clinically complete motor and sensory injury, ISNCSCI B classification refers to motor complete and sensory incomplete injury with preserved perianal sensation and/or anal sphincter contraction, and C and D refer to motor incomplete injuries. An E classification is normal. Several clinical studies have shown that the severity of injury, based on ASIA score is an important predictor of outcome after SCI.^{20,27,28} If an injury is complete (ASIA A) including a negative anorectal examination at 72 h post-injury, there is a low likelihood of return of substantial neurological recovery below the injury level, especially if the injury is in the thoracic region. The absence of perianal sensory function at one to two weeks post-injury has a strong negative predictive value for functional ambulation.²⁹ In contrast, patients with milder deficits (ASIA D) usually improve substantially. The recovery potential of the majority of individuals with severe incomplete injury (ASIA B and ASIA C) is quite variable but spontaneous neurological improvement is observed in as many as 60–80% of such patients.^{13,30,31}

The autonomic system has a critical role in physiologic homeostasis; its component neurons and pathways are damaged after SCI resulting in acute hypotension and bradycardia in severe injuries, followed by chronic positional hypotension and possible autonomic dysreflexia for injuries above the T6 level. Until recently, the functional status of the autonomic system has not been a routine component of SCI classification. There are now ISNCSCI reporting standards that extend the description of SCI to include autonomic function.³² Few studies have examined the relationship between acute measures of SCI severity and the recovery of autonomically-mediated function, such as bladder emptying.³³ Autonomic dysfunction is a major contributor to complications after SCI.

Ideally, methods for assessment of SCI within clinical trials would be robust, reproducible, and sensitive, as well as measure the outcome most relevant to the independent variable. The ISNCSCI classification was not developed as a method to stratify patients for enrollment to neural repair research studies or to assess the impact of such strategies.³⁴ Its measurement characteristics are ordinal, non-linear and limited by the relatively subjective ranges of motor and sensory scoring in those with incomplete injury,³⁵ precluding interval measurement, acquisition of storable data for quality assurance, and the requirement of considerable training to become

proficient.³⁶ The prognostic significance of the neurological level on the pinprick exam is greater than that for the light touch exam and may be a more sensitive indicator of the severity of SCI.³⁷ The motor exam does not include trunk muscle assessment; therefore, assessments of thoracic injury levels are based only on sensory scores. This can result in impairment grade changes that do not reflect meaningful recovery for the subject.^{38,39} The positive and negative predictive values of being classified as ASIA B or C on ambulation at one year are weak.⁴⁰ ASIA impairment grades result in assessments of variable resolution and groupings of heterogeneous populations with different individual recovery potentials.^{13,31,41–43} This results in large variations from the mean on outcome measures and a need to enroll a large number of patients in clinical trials.^{13,44}

Methods To Assess SCI Severity

MRI as a measure of acute injury severity

Magnetic resonance imaging (MRI) revolutionized the care of patients with SCI.⁴⁵ Acute post-injury MRI sequences allow visualization of the location and general severity of SCI by detecting edema and blood products within the spinal cord, the presence of ongoing compression, ligamentous and disc injury and displacement of the vertebral column. MRI is useful both to classify SCI severity and predict outcome^{7,46} based on the presence of hemorrhage, extent of edema and severity of initial compression. Hemorrhage is a strong indicator of the injury severity; a significant intraspinal hemorrhage (>1 cm length) is associated with a poor prognosis for neurological recovery.^{27,28,47} One to two levels of diffuse edema, as observed in a large subset of SCI patients, encompasses a wide spectrum of injury severity with >3 cm of longitudinal T2 signal change being associated with a poor recovery.⁴⁸ A normal initial MRI is associated with full recovery. Recent reports indicate that the rostral-caudal length of T2 edema signal increases over time⁴⁹ in the acute phase of injury and may be a marker of the severity of spinal cord compression related injury.⁵⁰ The placement of surgical hardware can create MRI artifacts that preclude visualization of the spinal cord injury site limiting the subsequent use of advanced imaging techniques. Although MRI can be obtained in many SCI patients, it may be contraindicated in those who are hemodynamically unstable or have implanted metallic devices.

Chronic injury

In subjects with chronic incomplete SCI (ASIA D), the extent of atrophy at the C2 level on T1 images is correlated with the ASIA motor score (left-right cord width) and with the ASIA pinprick and light touch scores (anterior-posterior cord width), and both motor and sensory measures correlated with spinal cord area.⁵¹ Atrophy was presumably due to tract degeneration. This suggests that spinal cord measurement after chronic injury at a single rostral level above the injury site may correlate with the extent of injury. It is generally easier to obtain high quality MR images from the C2 level than from lower cervical and thoracic levels.

Diffusion tensor imaging

In experimental studies of SCI, white matter preservation as determined histologically is highly correlated to the extent of neurological deficit and recovery.^{5,52–55} It would be useful for prognosis and possibly as a biomarker of response to therapy if

human spinal cord imaging could accurately assess the extent of damage to white matter. Diffusion MRI is a technique to visualize the extent and direction of movement of water molecules in tissue. In axonal pathways of white matter, water flow is constrained to the direction of the fibers (anisotropic), with a fractional anisotropic value approaching 1.0 (fully aligned). In the brain, diffusion tensor imaging (DTI) allows excellent visualization of normal white matter structure.⁴ Advances in diffusion-weighted imaging and algorithms for voxel-based tractography have further helped define the anatomy and configuration of intact and injured white matter tracts.⁵⁶ The application of DTI for the evaluation of the spinal cord has been challenging secondary to the small size of the spinal cord and significant artifact from the surrounding bone, respiratory and cerebrospinal fluid (CSF) motion, and the presence of edema after SCI. Several studies have applied DTI to the damaged spinal cord in persons with chronic spinal cord disease. In recent studies of cervical spondylotic myelopathy (CSM), changes in anisotropy were more accurately correlated to clinical symptoms amongst subjects than changes in standard MRI sequences^{57–59} and were more sensitive to early pathological changes.⁶⁰ In addition, baseline fractional anisotropy (FA) was correlated to whether improvement occurred or did not occur after surgical decompression.⁶¹ These CSM studies established the utility of DTI in the presence of spinal cord compression where resolution is especially challenging. The measurements from DTI and their reconstruction as color maps, show changes in the structure of spinal tissue that cannot be discriminated on standard T2 imaging (see Fig. 1).

In subjects with chronic SCI, Ellington and colleagues combined DTI with a “fuzzy interference system” to discriminate white and grey matter in the injured cervical spinal cord more accurately than standard images.⁶² Chang and colleagues studied 10 chronic SCI subjects and an uninjured control group and found that the absolute FA and apparent diffusion coefficient (ADC) values at the injury epicenter were correlated with the extent of preservation of neurological function.⁶³ In another study of 25 chronic SCI patients and 11 healthy controls, a correlation between FA and longitudinal diffusivity was seen at the injury epicenter in both hemorrhagic and non-hemorrhagic patterns of blunt SCI.⁶³ However, these values were correlated with the ASIA score only in patients with non-hemorrhagic SCI. In other subjects with chronic SCI, DTI was found to correlate with both clinical and electrophysiological measures of SCI completeness.²² Thus, DTI is promising to correlate the structure of the injured spinal cord with neurological outcome.

In acute SCI, DTI can measure the extent of spinal cord disruption both regionally and at the injury epicenter, and detect changes in ADC at segments rostral and caudal to the epicenter that are not apparent on T2-weighted images.⁶⁴ A study of subjects with acute incomplete SCI found a correlation between ADC and neurological scores at admission and recovery and also predicted whether T2 signal changes on acute MRI would evolve to cavitation. The authors argue that a low ADC is associated with cytotoxic edema associated with cellular energy failure.⁶⁵ DTI has been applied in studies of acute SCI in pediatric subjects where the clinical exam may be especially challenging. DTI measures were correlated to the results of clinical testing of the sacral segments to determine completeness or incompleteness.⁶⁶ Another study that compared T2 and diffusion weighted signal changes in acute SCI subjects did not find that diffusion-weighted images were more sensitive to detect signal abnormalities, however diffusion tensor calculations were not apparently performed.⁶⁷ Future developments that

improve spinal cord DTI may lead to the ability to quantify tract injury, endogenous repair, and the effect of therapeutics such as myelin reparative cells.

Magnetization transfer (MT) imaging is a technique to discriminate the difference in resonance properties of hydrogen molecules in tissue that are bound to macromolecules such as myelin from hydrogen molecules of water.^{68,69} The ratio of magnetization transfer in the free and macromolecular pools is correlated to the quantity and integrity of the macromolecular structures and can be used to detect myelin and axonal pathology.⁷⁰ The combination of diffusion-weighted and MT imaging generated data that correlated well with the motor and sensory scores of subjects with chronic SCI.⁷¹ In longitudinal studies, the MT ratio may be useful to detect demyelination, axonal damage, and remyelination.^{72,73}

Magnetic resonance spectroscopy (MRS)⁷⁴ has been used to study the spinal cord and brain of persons with chronic SCI. Changes in the concentration of metabolites found within the anterior cingulate and the thalamus correlated with chronic neuropathic pain.^{75–76} In an MRS study of subjects with CSM, the N-acetyl aspartate/choline (NAA/Cho) ratio was reduced indicating chronic neuronal loss and lactate peaks were found in several patients consistent with ongoing spinal cord ischemia.⁷⁷ In a subsequent study using proton MRS, the authors correlated the NAA/Cho ratio with reduced neurological function in CSM patients.⁷⁸ There is continuing development of improved methods for spinal cord MRS to increase the signal to noise ratio.⁷⁹ An MR technique called chemical exchange saturation transfer (CEST) recently has been used to demonstrate differences in glutamate concentrations in gray and white matter of the normal spinal cord.⁸⁰ Functional MRI (fMRI) also is showing promise to confirm the presence of post-injury connectivity in SCI²¹ and to validate clinical tests of SCI completeness such as the cortical response to deep anal pressure (minimum definition of ASIA B).⁸¹ In addition, fMRI can demonstrate alerted signal processing of thermal stimuli within the spinal cord. Patients who experience recovery of sensation have an increased number of loci of activation within the spinal cord grey matter than normal controls.⁸² Cortical changes studied longitudinally over one year using fMRI were correlated with the extent of neurological recovery.⁸³ Thus, several MR-based techniques are now useful to show structure, physiology, and connectivity and plasticity of the spinal cord.

Physiologic measurement of SCBF after SCI

Normally, spinal cord blood flow (SCBF) is tightly regulated to the metabolic requirement of spinal cord tissue.⁸⁴ The spinal cord has a very limited tolerance to ischemia⁸⁵ with similar critical flow levels as the brain (18–20 mL/100 g/min). Immediately after SCI, autoregulation of blood flow is lost^{86–88} and either hyperemia or low flow may occur depending on injury severity. Currently, no validated methods are in clinical practice to assess SCBF in real time in the clinical SCI setting. From both a clinical physiology and therapeutic perspective, the development of accurate real-time monitoring tools similar to what is available for brain monitoring could be very useful. The lack of a viable clinical technique to measure SCBF in the acute SCI setting has led to clinical practice recommendations that seek to maintain MAP at a level consistent with reasonable SCBF (MAP > 80/85 mm Hg).^{89,90} The evidentiary basis for this practice has been questioned^{91,92} and high blood pressure in the setting

FIG. 1. Pictorial depiction of multiple modalities for pathogenesis-based severity assessment after acute spinal cord injury. Derived from Savic and colleagues (2007); Dray and colleagues (2009)¹⁴²; Chang and colleagues (2010); Iyer and colleagues (2010)¹⁴³; and Farrar and colleagues (2011)¹⁴⁴, with permission of the publishers. Color image is available online at www.liebertpub.com/neu

of the disrupted blood brain barrier may be harmful.⁹³ Laser Doppler flowmetry has been employed in preclinical studies to quantitatively measure the blood flow in the spinal cord.^{94–96} This method allows for measurement of blood flow using small probes placed over the dura at the spinal segment of interest. The clinical application of this technique remains challenging because it requires surgical insertion of the probe in the acute phase after SCI. In other studies, a clinically available monitoring sensor that measures pH, partial pressure of oxygen, and carbon dioxide has been inserted into the thecal space to measure CSF oxygenation during distal aortic clamping in a pig model.^{97,98} A non-invasive method

using transcutaneous near-infrared spectroscopy⁹⁹ to measure oxygen saturation of the spinal cord could detect ischemia in a pig model but the spatial resolution of this technique to detect focal ischemic change has not been assessed.

Neurophysiological assessment techniques in SCI

The conventional neurological examination has limitations and is susceptible to subjectivity that is affected by the clinician's level of training.^{17,43} In multisystem trauma, the neurological examination is further complicated if there is head injury,

extremity fractures and casts, peripheral nerve injury, preexisting neurological disease such as neuropathy, and obtunding medications or drugs. Evoked potentials (EP) are measures of neural activity that are induced by supraphysiologic stimuli of nerves or CNS structures. When applied properly, they can assess conduction in specific spinal tracts as a measure of their physiological integrity, even in unconscious patients. Although many acute severe SCI patients lack detectable evoked potentials, EPs could be used to assess and monitor spinal cord conduction in those with incomplete injury. The presence and amplitude of EPs fluctuates with spinal cord perfusion.¹⁰⁰ However, EPs are generally not used in the acute resuscitation setting prior to anesthesia for decompression and fixation surgery. Contributing factors that make acute electrophysiological assessment difficult are the electrical interference in intensive care unit (ICU) settings and the stimulation intensities required to detect control waveforms in the presence of SCI that may be painful in the unanesthetized patient. In addition, there is a small amount of abrupt neck motion associated with motor evoked potentials (MEP) induction. During anesthesia, it is much easier to conduct good quality neuromonitoring. MEPs are obtained by stimulating the cortex and recording from muscles that act as natural signal amplifiers. Somatosensory evoked potentials (SSEPs) are elicited by repetitive nerve stimulation and recorded from the cortex after filtering to subtract non-specific cortical activity and electrical noise. MEPs and SSEPs are valuable to detect possible adverse intra-operative changes due to hypotension, over-distraction, occult nerve and cord compression, or misplacement of a device such as a pedicle screw during spinal surgery.¹⁰¹

After SCI, the presence of retained MEPs correlates with a prognosis for improved motor function.¹⁰² The presence or absence of reproducible MEPs is more important prognostically than their latency and amplitude. Retention of even small amplitude ulnar nerve SSEPs in the subacute phase after SCI is highly correlated to recovery of hand function.^{103,104} The presence of a detectable tibialis anterior MEP within 15 days of injury is strongly linked to the extent of motor score improvement, walking recovery, and conversion of ASIA grade to D status.^{105,106} The presence of retained evoked potentials has not been correlated with the quantity of preserved neural tissue. It is not known how few axons are sufficient to support detectable signal conduction. Many factors influence the presence or absence of evoked potentials such as temporal summation of action potentials.

Level of injury

EPs can provide objective assessment of injury level,¹⁰⁷ establish the dermatomal levels at which sensory function is preserved, and measure change over time and provide quantitative data. This is especially useful at thoracic levels where a segmental motor examination is lacking. For this purpose, quantitative sensory testing (QST), dermatomal SSEPs (dSSEPs), and contact heat evoked potentials (CHEPs) have been investigated.¹⁰⁸ Quantitative Sensory Testing (QST)^{109,110} involves administration of three types of stimuli to determine the threshold of detection of electrical, thermal and vibratory stimuli at the ASIA key sensory points in different dermatomes. This testing is based on the subject's report of perception differing from EP testing that measures evoked signals. This distinction is important because the patient's report of sensation is less artificial than tests that use supraphysiologic stimuli to generate an evoked cortical waveform and that

are not specific to a type of physiologic receptor such as vibratory and thermal receptors.

QST data can be compared with normograms obtained from control populations and pictorially depicted to clearly show the level of injury, hyperesthesia, hypoesthesia, or zone of partial preservation. Serial performance of QST can be used to monitor neurological change and treatment effects. In a prospective evaluation of an SCI cohort, QST was more sensitive than the standard neurological testing.¹⁰⁹ When combined, the three modalities (electrical/light touch, temperature and vibration) test both the dorsal column (light touch and vibration) and spinothalamic (temperature) sensory inputs. The stimuli are very reproducible and are selected to assess both small unmyelinated afferents and also larger myelinated afferents. Recently, a small prospective crossover trial evaluating the effects of transcranial magnetic stimulation (TMS) reported consistent post-TMS changes in sensory thresholds detectable with (electrical perceptual threshold) EPT testing¹¹¹ indicating its sensitivity. Another clinical study reported good reliability of QST on repeated examinations at one, three, and six months after SCI.¹¹² Before establishing widespread use, large clinical studies should include this outcome measure to study the reliability of this technique in the acute phase after SCI. The predictive value of early QST assessment for long-term neurologic outcomes also is largely unknown.

It is very important to understand that the subject's tolerance is a limiting factor in conducting these studies. Most studies have been performed in subjects with chronic SCI who have volunteered, and in some instances have been compensated. Patients with acute injuries, and in the first weeks post-injury have limited tolerance to extended durations of testing.

Measures of spinal cord grey matter integrity

SSEPs and MEPS mainly evaluate axonal tract conduction. It is also extremely important to evaluate for the presence of functional grey matter at and near the injury level. Peripheral nerve stimulation tests such as H reflexes and F waves can be elicited to verify the integrity of the reflex arc and motoneurons respectively.¹⁹

Contact heat evoked potentials (CHEPs). CHEPs assess the integrity of the spinothalamic tract based on the stimulation of small diameter afferents that convey thermal information, especially of noxious quality. In some SCI patients, a positive CHEPs may be elicited in a dermatome that lacks pin prick sensation on ASIA testing.¹¹³ CHEPs are also useful in the assessment of neuropathic pain.¹¹⁴

dSSEPs. Surface electrodes are placed over successive rostral to caudal dermatomes and the impedance tested and minimized. Then square wave pulses similar to those for nerve stimulation are applied and a scalp SSEP recorded.¹¹⁵ This technique assesses the integrity of large diameter posterior column axons. Dermatomal SSEPs, electrical perceptual testing, QST and ASIA pinprick and light touch testing may yield differing results in dermatomes at and below the lesion level. This is because of the differing stimulation methods, the use of physiologic or supraphysiologic stimuli, and the presence or absence of residual conducting axons. The benefit of such testing is its sensitivity and potential to quantify small changes. However, there are few longitudinal studies to allow an evaluation of how these measures change during recovery from acute SCI.

Quantitative electromyography (EMG). Neurorestorative treatments are more likely to be effective when some spared neurons and axons capable of plasticity are proximate to and pass through the lesion epicenter. There is now considerable evidence that some individuals classified as having a motor complete lesion can voluntarily elicit “subclinical” motor activity detected using EMG below their lesion level.^{116–118} Further, the subclinical EMG changes can precede clinically observable muscle activity during recovery from SCI.¹¹⁹ This testing might be very useful in therapeutics trials to detect changes in motorneuronal activity that are below the level of clinical detection.

Intercostal motor evoked potentials. The motor output of the ventral horn neurons of the thoracic spinal cord includes intercostal muscles and sympathetic ganglia. Careful neurophysiologic recordings can discriminate normally innervated intercostal muscles and those that are denervated.¹²⁰ This is another method to objectively establish the level of injury.

Autonomic testing. Testing of the integrity of the autonomic system involves assessment of postural vascular responses (heart rate/blood pressure) to hypotension (tilt table), sympathetic skin responses¹²¹ that assess impedance changes due to variation in sweating, and axonal flare responses.¹²² It is possible to be ASIA incomplete but lack detectable sympathetic function.¹⁷ Two of the most problematic complications of SCI, autonomic dysreflexia and postural hypotension are due to loss of supraspinal regulation of sympathetic activity.

Molecular Markers of Injury Severity

In several diseases, the quantification of surrogate markers from blood has proven very useful in diagnosis, treatment, and assessment of response to treatment. Classic examples are cardiac enzymes, liver enzymes, and lipid and cholesterol levels. For neurological diseases, the accumulation of proteins that are normally intracellular can be measured from CSF or blood. In Alzheimer’s disease, CSF tau and beta amyloid levels are predictive markers of the presence of the disease and risk of progression.¹²³ Several studies have assessed whether the presence and concentration of neuronal and glial proteins released as a result of brain and spinal cord trauma correlate with the severity of clinical injury. These biomarkers include structural components of neurons and glia, their breakdown products, and cytokines whose expression is increased in response to injury.¹²⁴ An important finding has been that ASIA A injuries are associated with much higher levels and variability of biomarker concentrations than ASIA B and C injuries validating the concept that more severe clinical injuries are associated with greater damage, and that neurologically complete injuries vary in their structural severity. Thus, some ASIA A injuries cause massive destruction while others may only exceed the threshold of injury associated with clinically completeness. These less severe but complete injuries may be more effectively treated with neuroprotective and neurorepair strategies. The molecules that appear most useful as SCI biomarkers are those that are specific to the CNS, and can be detected in serum, as this is less invasive than CSF sampling.¹²⁴ Proteins studied have included neurofilament (NF), glial fibrillary acidic protein (GFAP), myelin-basic protein (MBP), S-100 β tau, and spectrin breakdown products.¹²⁴ Inflammatory cytokines of interest have included interleukin 6 (IL-6), IL-8, monocyte chemoattractant protein-1 (MCP-1), tumor

necrosis factor alpha and IL-1. It is also anticipated that some of these biomarkers may be useful to detect a response to treatment,¹²⁵ although that has not yet been clarified in clinical subjects.

The biomarker phosphorylated neurofilament H (pNF-H) was found to correlate with the extent of brain injury in experimental models.^{126,127} In pediatric brain injury, high levels of the biomarkers S-100 β , GFAP, neuron-specific enolase, and NF-H correlated with the probability of mortality.¹²⁸ Serum and CSF biomarkers have been combined with clinical data in a multivariate predictive model of outcome after brain trauma.¹²⁹

Cerebrospinal fluid biomarkers of injury severity

It is important to establish reproducible quantification methods for biomarkers, learn their temporal evolution after injury and optimize sampling time-points to make studies more efficient and feasible. Studies in SCI patients have shown much higher levels of injury biomarkers in those with complete versus incomplete injury. In a study by Hayakawa and colleagues, the quantity of phosphorylated NF-heavy chain (pNF-H) was measured from serum in a cohort of patients with cervical SCI of complete and incomplete AISA impairment grades.¹³⁰ Blood samples were collected at 6, 12, 18, 24, 48, 72 and 96 h, and six, eight, 10, 14 and 21 days after the injury. The average blood level at 18 h in those with ASIA A injury was 5280 pg/mL and for incomplete subjects 449 pg/mL. In the one ASIA A subject that converted to AIS C within a five-month follow-up period, the serum level was much lower than other ASIA A subjects who did not convert. This small study is promising for possible use of pNF-H to identify those ASIA A subjects with a higher probability to convert to a higher grade. In another study that examined pNF-H levels in rats randomized to placebo (saline) or minocycline after acute SCI, lower levels of pNF-H detected in the treated group were associated with better locomotor scores, although the effect size was modest.¹²⁵ This study indicates a potential role for pNF-H as a biomarker for treatment effect in acute neuroprotection studies. In other studies, tau was found to correlate with injury severity in acute human SCI.^{131,132} In a study by Kwon and colleagues,⁴⁵ the levels of the injury biomarkers IL-6, IL-8, MCP-1, tau, S100 β and GFAP measured from CSF after acute SCI fell to control levels within 72 h post-injury. The mean levels of IL-6, MCP-1, tau, S100 β , and GFAP 24 h post-injury differed significantly for initial impairment grades A, B and C. In a combined model from the same study, S100 β , GFAP, and IL-8 showed a positive predictive value of 89% to predict the ASIA grade at 24 h, and were correlated with the extent of upper extremity motor score recovery six months after SCI. Further investigation into the utility of these and other biomarkers for injury severity assessment, prognostication of neurological recovery, and response to therapy of human SCI is needed.

Impact of genetic variation on recovery potential

Genetic variability may influence injury severity and recovery. Single nucleotide polymorphisms (SNPs) in the ApoE allele have been associated with differences in motor recovery after SCI.¹³³ In a study by Guimaraes and colleagues, SNPs were found to be very frequent in both coding and non-coding regions. This study assessed the frequency of the polymorphisms *ALOX12*, *APOE*, *BDNF*, and *NINJ1* in persons with and without SCI and found a

total 95,276 SNPs across 66 subjects for the five genes.¹³⁴ In another study, the presence of a nonsense SNP in alpha-actinin-3 allele in persons with SCI was associated with paralyzed muscles that lacked Type II fibers. This muscle phenotype could reduce recovery potential.

SCI outcome models. For cervical SCI, a combination of clinical and radiographic assessment with electrophysiological studies improved the prediction for future recovery.¹⁰³ Zorner and colleagues, using data within 16–40 days after injury from the European multicenter study about spinal cord injury (EM-SCI),¹³⁵ tested multivariate models that included age, sex, ASIA Impairment Scale (AIS), injury level, subacute upper and lower extremity motor scores, ASIA sensory scores, and SSEPs in 55 combinations to determine the factors most predictive of locomotor recovery on two well standardized walking measures, the 6-min walk test and the Walking Index for Spinal Cord Injury II. They found that the best predictors differed between those with tetraparesis and those with paraparesis and developed a hierarchical predictive algorithm that could be used to stratify patients into those likely and not likely to become dependent or independent functional walkers.⁴⁰ A study from the same group found that sacral sparing could be predicted by S1 motor and sensory preservation or motor and sensory preservation more than three levels below the neurological level indicating that the ISNCSCI data can be correlated in more complex ways than was understood previously.¹³⁶ Wilson and colleagues used data from the North American Clinical Trials Network and the Surgical Treatment of Spinal Cord Injury Study to develop a predictive equation that assigned weights to age, AIS grade, ASIA motor scores, and MRI presence of spinal cord blood or edema to predict the Functional Independence Measure and the probability of independence at one year post injury.¹³⁷ Thus, multivariate models to predict neurological outcome are being established from clinical, biomarker, radiologic and electrophysiological data. A great deal of effort has been directed to develop methods to predict neurological recovery. It should be borne in mind that the correlation between ASIA impairment grades with independence and life quality as measured with the spinal cord independence measure (SCIM)¹³³ or functional independence measure (FIM)¹³⁸ is not especially strong. However, SCIM and FIM correlations are higher with ASIA motor and sensory scores and specific functions such as shoulder motion.¹³⁹

SCI outcome heterogeneity in prior studies

An especially striking example of the heterogeneity of outcomes that we have discussed in this review is evident in the recently reported Phase 2 study of minocycline in acute SCI by Casha and colleagues.¹⁴⁰ In this study, the enrolled subjects were stratified as motor complete (ASIA A/B, motor incomplete (ASIA C/D), and central cord injury, and randomized to receive high dose, low dose, and placebo with 12 h of SCI. The subjects were treated in a single center with uniform treatment standards. The pharmacokinetics of minocycline were uniform in both the dose groups. Despite this, the neurological outcomes showed substantial variation within the three stratified groups. For example, in the placebo group, the motor score recovery had a standard error of the mean that is 70% of the mean recovery score. The standard deviations of motor score values in the cervical complete and incomplete groups were 15.8 and 19.3, respectively. Thus, the demonstration of a significant treatment-related outcome would

require a study with a larger enrolment as the authors concluded. This study indicates the difficulty of designing acute neuroprotective studies even with some *a priori* stratification of enrolled subjects. Perhaps additional imaging or biomarker data could allow further injury stratification, or serve as exclusion criteria to predict non-responders.

Conclusion

SCI care continues to improve. Now that clinical trials of neuroreparative agents are being tested, it is increasingly important to have valid methods to predict who may benefit from these therapies, measure their bioactivity, and their neurological and other effects.^{6,119,141} We reviewed both the early prognostication and outcome measure sides of this equation. For prognostication, in addition to current clinical measures, certain methods are more feasible than others within the early post-injury time frame. Acquisition of diffusion-weighted tensor MRI sequences is feasible during acute post-SCI MRI and may provide quantitative information about the integrity of white matter. Serum and/or CSF biomarkers also are feasible and may provide both an index of injury severity and response to therapy. Diffusion-based MRI, magnetization transfer, and biomarker measures may detect those injuries that lack repairable axons such that certain experimental treatments (e.g., cell transplantation for myelin repair) would be futile. Detection of preserved evoked potentials within the acute and subacute time frame are highly significant for neurological recovery. Further development of genetic assessments may allow for description of subjects with greater or lesser recovery potential to be detected. This may substantially aid in interpreting the variability in outcome after SCI and treatment. Combinations of the assessments we have discussed in this review may increase our ability to correlate injury data and outcome.

Finally, we reviewed the need for methods to assess the physiology and metabolic state of the injured cord to optimize tissue preservation. No such methods are currently in use and their development is important.

We have concluded that improvements in the assessment of spinal cord injuries using advanced imaging techniques may improve the ability to predict neurological outcome, rationally enroll subjects to reparative clinical trials, and interpret treatment effects. The detectable products of neural tissue injury and inflammation are promising to provide a window into the temporal evolution of SCI, its biology, and to serve as surrogate markers of the effects of neuroprotective treatments. Current methods are not sufficient to optimize neural preservation after injury. The development of accurate responsive physiologic monitoring devices that could evaluate spinal cord tissue oxygen levels and blood flow is needed. Advanced imaging methods, such as fMRI and neurophysiological methods that focus on segmental plasticity, may be able to provide new insights into the evolution of neural connectivity after injury.

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Author Disclosure Statement

No competing financial interests exist.

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