Metabolites of neuroinflammation relate to neuropathic pain after spinal cord injury

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Disclosure

The authors report no disclosures relevant to the manuscript.
Glossary

AIS = ASIA Impairment Scale

ASIA = American Spinal Injury Association

ISNCSCI = International Standards for the Neurological Classification of SCI

MRI = magnetic resonance imaging

MRS = magnetic resonance spectroscopy

NP = neuropathic pain

TE = echo time

TR = repetition time

SCI = spinal cord injury

T1w = T1-weighted

T2w = T2-weighted
Abstract

Objective:
To determine whether cervical cord levels of metabolites are associated with pain sensation after spinal cord injury (SCI), we performed magnetic resonance spectroscopy in SCI patients with and without neuropathic pain (NP).

Methods:
Cervical cord single-voxel spectroscopic data of 24 SCI patients (14 with NP, 10 pain-free) and 21 healthy controls were acquired at C2/3 to investigate metabolite ratios associated with neuroinflammation (choline-containing compounds to myo-inositol (\(t\text{Cho} / mI\)) and neurodegeneration (total N-acetylaspartate to myo-inositol (\(t\text{NAA} / mI\))). NP levels were measured and Spearman’s correlation tests assessed associations between metabolite levels, cord atrophy, and pin-prick score.

Results:
In patients with NP, \(t\text{Cho} / mI\) levels were increased (\(p=0.024\)) compared to pain-free patients and negatively related to cord atrophy (\(p=0.006, r=0.714\)). Better pin-prick score was associated with higher \(t\text{Cho} / mI\) levels (\(p=0.032, r=0.574\)). In pain-free patients, \(t\text{Cho} / mI\) levels were not related to cord atrophy (\(p=0.881, r=0.055\)) or pin-prick score (\(p=0.676, r=0.152\)). \(t\text{NAA} / mI\) levels were similar in both patient groups (\(p=0.396\)) and were not associated with pin-prick score in patients with NP (\(p=0.405, r=0.242\)) and pain-free patients (\(p=0.117, r=0.527\)).

Conclusions:
Neuroinflammatory metabolite levels (i.e. \(t\text{Cho} / mI\)) were elevated in patients with NP; its magnitude being associated with less cord atrophy and greater pain sensation.
(e.g. pin-prick score). This suggests that patients with NP have more residual spinal tissue and greater metabolite turnover than pain-free patients. Neurodegenerative metabolite levels (i.e. $tNAA/mI$) were associated with greater cord atrophy, but unrelated to NP. Identifying the metabolic NP signature provides new NP treatment targets and could improve patient stratification in interventional trials.

**Classification of Evidence:**

This study provides Class II evidence that levels of MR-spectroscopy-identified metabolites of neuroinflammation were elevated in SCI patients with NP compared to those without NP.
Introduction

Spinal cord injury (SCI) is a life-changing event which generally leads to sensorimotor dysfunction below the injury level\(^1\). Neuropathic pain (NP) arises as a secondary complication in more than 50% of the SCI population\(^2\) and has a negative impact on life quality\(^3\). NP generally emerges within several months after SCI\(^4\) and is paralleled by trauma-induced neuroinflammation and neurodegeneration eventually affecting the entire neuraxis\(^5\)\(^-\)\(^7\). However, NP-related metabolite level changes underlying neuroinflammatory and neurodegenerative changes are understudied, especially in the injured spinal cord.

Magnetic resonance spectroscopy (MRS) can non-invasively quantify metabolite levels\(^8\)\(^,\)\(^9\) reflecting a biochemical profile of neuroinflammatory\(^10\) (i.e. elevated choline-containing compounds (\(t\text{Cho}\)), cell membrane and myelin turnover marker\(^11\) and myo-inositol (\(mI\)), glial cell marker\(^12\)\(^,\)\(^13\) and neurodegenerative\(^14\) (i.e. decreases in total N-acetylaspartate (\(t\text{NAA}\)), neuronal cell integrity marker\(^14\)) processes. In SCI patients with NP, higher \(t\text{Cho}\)\(^,\)\(^,\)\(^15\)\(^,\)\(^16\) and \(mI\)\(^,\)\(^,\)\(^,\)\(^15\)\(^-\)\(^,\)\(^18\) were identified in key brain areas of pain processing compared to pain-free patients. In contrast, \(t\text{NAA}\) was decreased to a similar extent and unrelated to NP\(^15\). In the injured cervical cord, Wyss et al.\(^9\) observed trauma-induced reductions in \(t\text{NAA}\) and \(t\text{Cho}\) and elevated \(mI\). Crucially, these neurodegeneration and neuroinflammation related changes showed clinicopathological relationships.

However, how metabolic changes associated with neuroinflammation and neurodegeneration in the cervical cord relate to the presence of NP in SCI patients is understudied. This study therefore aimed to investigate by means of spinal MRS metabolic changes at cervical level C2/3 and their clinicopathological associations in chronic SCI patients with NP and pain-free SCI patients.
Subjects and methods

Standard Protocol Approvals, Registrations, and Patient Consents

The local ethics committee of Zurich approved the study protocol (KEK-ZH-No. 2014-610, PB_2016-00126, PB_2018-00937) which was conducted in accordance with the Declaration of Helsinki. All participants of this study were informed prior to study enrolment about the aim and procedure and provided written informed consent. A subset (9 paraplegic patients, 9 tetraplegic patients, and 11 healthy controls) of the data of this study was previously reported to assess metabolite ratios in the cervical spinal cord after SCI\(^9\).

Primary research question

The primary research question of this study was if levels of metabolites of neuroinflammation assessed by cervical cord MR-spectroscopy are elevated in SCI patients with NP compared to those without NP.

Classification of evidence

This manuscript describes a diagnostic accuracy study which provides Class II evidence that levels of MR-spectroscopy-identified metabolites of neuroinflammation were elevated in SCI patients with NP compared to those without NP.

Participants

Twenty-four SCI patients and 21 healthy controls were recruited between March 2016 and September 2018. SCI patients fulfilled the following inclusion criteria: 1) chronic traumatic injury (>1 year post-injury), 2) no other neurological or mental disorders, 3) MRI compatible. Exclusion criteria of the study participants including healthy controls were preexisting neurological, mental, or medical disorders affecting the outcome. One patient had to be excluded due to scan artifacts and another one because he did not fit into the neurovascular coil.

Experimental design
MRI protocol

All study participants underwent magnetic resonance (MR) measurements on a 3T Philips scanner (Achieva, Release: 3.2.3, Philips Healthcare, Best, The Netherlands) using a 16 channel SENSE neurovascular coil (Philips Healthcare, Best, The Netherlands). Spectra were acquired from the cervical spinal cord at level C2/3 (i.e. above the level of injury for all, but one patient). The participants lay in the scanner in a head first supine position and the total scan duration was approximately 45 minutes. MR measurement sequences included a survey scan, anatomical T1-weighted (T1w) and T2-weighted (T2w) scans, and spectroscopic measurements. At spinal level C2/3, T2w images (TR: 3000 ms, TE: 120 ms, flip angle: 90°, in-plane resolution: 0.5 mm x 0.5 mm, slice thickness: 3.2 mm) were used to place the spectroscopic voxel (dimensions: 6 mm x 9 mm x 35 mm, 1.9 ml). The metabolite cycling (MC) technique was then applied in combination with inner volume saturated PRESS using broad-band outer volume suppression pulses with optimal flip angles as reported previously. In addition, we used a second-order projection-based shimming routine. To reduce patient motion to a minimum, the spectroscopic acquisition was split into measurement acquisition blocks of 128 or 256 signal averages and voxels were re-adjusted based on an updated T2w image (TR: 2000-2500 ms (heart beat triggered), TE: 30 ms, number of total signal averages (NSA): 512, spectral bandwidth: 2000 Hz, readout duration: 512 ms).

MRI post-processing

Quality of MR spectroscopy measurements

The spectroscopic measurement blocks were checked for motion artefacts. No measurement block had to be excluded. Metabolic concentration values could be
determined for all metabolites ($t_{\text{Cho}}$, $t_{\text{NAA}}$, and $mI$) in all measurements.

Representative planning images and spectra are shown for a healthy control, a pain-free SCI patient, and a SCI patient with NP (Fig 1). An oversimplified overview of the molecules measured and the presumed interactions is illustrated in Fig 2.

**Post-processing and quantification of spectroscopic data**

We used MATLAB 2014b (MathWorks, Inc., Natick, MA, USA) and the commercially available MRecon framework (version: 3.0.530, GyroTools LLC, Zurich, Switzerland) for reconstruction of the spinal cord spectroscopic data from the raw data of the scanner. Eddy current, phase and frequency alignment parameters were extracted from the unsuppressed water spectrum reconstructed from the MC series. Subsequently but before merging all acquisition blocks eddy current correction and frequency alignment were applied to the metabolite spectra reconstructed from the MC sub-series by an ad-subtract scheme as previously reported\(^\text{19}\). Before quantification, truncation and zero filling was used in the time domain after 200 ms. Relative values of metabolite concentrations were obtained by scaling them to myo-inositol (as previously reported\(^\text{9}\)), since absolute quantification by internal spinal water referencing is not reliably feasible due to the pulsating surrounding cerebrospinal fluid (CSF) as previously shown in supplementary Figure 1 of Wyss et al.\(^\text{9}\). Spectroscopic data of the cervical spinal cord were fitted and quantified using LCModel\(^\text{26}\). A basis set for TE=30 ms was used including simulated basis set model data of N-acetylaspartate (NAA), N-acetyl-aspartyl-glutamate (NAAG), glutamate (Glu), glutamine (Gln), glycerophosphocholine (GPC), phosphocholine (PCh), creatine(Cr), scyllo-inositol (sI), and myo-inositol ($mI$). Strongly overlapping resonance lines required a combination of the spectra of the following metabolites: NAA + NAAG = $t_{\text{NAA}}$, GPC + PCh = $t_{\text{Cho}}$ and Glu + Gln = Glx. A spectral range of
0.4 to 4.0 ppm was used in the fitting settings. To prevent artificial cut-off effects introduced by a cut-off value of 20%\cite{27}, we included metabolic ratios with Cramer-Rao lower bounds (CRLB) smaller than 100%.

**Cross-sectional spinal cord area at cervical level C2/3**

We used Jim 7.0 (Xinapse Systems, Aldwincle, UK) to assess the cross-sectional spinal cord area (SCA) at cervical level C2/3 on T2w images in all healthy control participants and SCI patients. SCA was measured in three consecutive slices at the bottom of vertebra C2/3. In a first step, we determined a region of interest (ROI) in the middle of the spinal cord on axial slices. In a next step, we used an active-surface model\cite{28} for the automatic calculation of SCA. Last, we manually adjusted the SCA outline in those slices of study participants where automatic cord determination did not work properly (5 cases). Cross-sectional spinal cord area could not be calculated for one patient due to motion artifacts during the magnetic resonance (MR) acquisition.

**Clinical assessments**

All SCI patients were clinically assessed using a comprehensive clinical protocol including 1) the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) protocol for pin-prick and light-touch scores\cite{29} and 2) the European Multicenter Study about Spinal Cord Injury (EMSCI) pain questionnaire (V4.2, http://www.emsci.org/).

Using the ISNCSCI protocol, patients were classified as AIS A (i.e. complete injury. No sensory or motor functions preserved in sacral segments), AIS B, C, or D (i.e. incomplete injury), or AIS E (i.e. no functional impairment) according to the neurological classification of SCI, the American Spinal Injury Association (ASIA)
Impairment Scale (AIS). The neurological level of injury (NLI) is defined as the uppermost segment with neurologically intact motor and sensory scores.

In all SCI patients, the EMSCI pain questionnaire was used as a screening tool for pain after SCI. This pain questionnaire examines various aspects of pain (e.g. current pain intensity, average and maximal pain intensity during the last week, location and quality of pain, intensity of allodynia and paresthesia). The pain intensity was rated using an 11-point numeric rating scale with “0” indicating no pain and “10” indicating the worst imaginable pain. In addition, the type of pain was explored and grouped into nociceptive (i.e. musculoskeletal or visceral) or NP (i.e. at, below, or at and below the lesion level). To be classified as at-level NP, ongoing pain had to be experienced within the 3 dermatomes below the neurological level of injury (NLI), one dermatome above the NLI, or both. Below-level NP on the other hand was defined as NP more than 3 dermatomes below the NLI. All patients completed the full protocol.

Statistical analysis
Statistical analyses were performed using the R software package (version 3.4.3) and Stata software (version 14.2; StataCorp LP, College Station, TX) which was also used for visualization. We applied a one-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test for pairwise comparison of healthy controls, SCI patients with NP, and pain-free SCI patients regarding their age at the time point of measurement. Unpaired two-tailed t-tests were used to compare the pin-prick score and time since injury between patients with NP and pain-free patients. Between-group comparisons graphics of metabolic ratios show boxplots including the median and quartiles. These group differences between the healthy control group, SCI patients with NP, and pain-free patients were assessed for metabolite concentration ratios of $t_{Cho/ml}$ and $t_{NAA/ml}$ at spinal level C2/3 using a Kruskal–Wallis test.
followed by pairwise Mann–Whitney U tests. We used Spearman’s rank correlation tests to investigate associations between the amount of cervical cord atrophy in SCI patients, assessed by the cross-sectional spinal cord area (SCA) at cervical level C2/3, and metabolite concentration changes at the same level. Last, we analyzed relationships between clinical measures of pain perception (pin-prick score) and the metabolite ratio of \( \text{tCho}/\text{mI} \) at C2/3 using Spearman’s rank correlation tests. The confidence interval was set to 95%. Results with an uncorrected p-value of 0.05 or smaller were regarded as significant. We did not adjust for multiple testing to reduce type II errors which potentially increased type I errors. Age was included as a covariate of no interest in the statistical model to adjust for age dependency.

**Data Availability**

Anonymized grouped data will be shared by request from a qualified investigator.

**Results**

**Demographics and clinical characteristics**

Fourteen SCI patients with NP (12 men, age [mean±standard deviation]=52.2±10.5[y], years since injury=11.3±9.2), 10 pain-free SCI patients (10 men, age=50.0±10.3[y], years since injury=18.4±10.5), and 21 healthy control participants (18 men, age=46.0±11.2[y]) were recruited. From all SCI patients, 12 were classified as functionally complete (American Spinal Injury Association Impairment Scale grade (AIS) A) and 12 as functionally incomplete (AIS grade B-D) (Table 1). The NP group consisted of 8 tetraplegic and 6 paraplegic patients while the pain-free group consisted of 5 tetraplegic and 5 paraplegic patients. Pin-prick score was 64.3±31.9 and 46.9±20.6 for SCI patients with NP and pain-free patients,
respectively (p=0.145, 95% confidence interval (CI): -41.3 6.5). Patients with NP reported a mean pain intensity of 4.3 (standard deviation (SD) of 1.7, min=1, max=7) on a scale from 0 to 10. Mean age at injury was 40.9 years (SD of 14.0) for the SCI patients with NP and 31.6 years (SD of 13.5) for the pain-free SCI patients. The participants’ mean age did not differ between any of the groups (p=0.245). There was no significant difference in mean time since injury in years between the patient groups (p=0.092, 95% CI: -1.3 15.5).

Changes of choline-containing compounds to myo-inositol

We first confirmed that there was a difference in \( \frac{tCho}{mI} \) levels between groups (p=0.010) as previously reported for SCI patients and healthy controls in a subset of this population\(^9\). SCI patients with NP had a similar \( \frac{tCho}{mI} \) ratio (p=0.699, 95% CI: -0.049 0.029, Fig 3A) as healthy controls but an elevated \( \frac{tCho}{mI} \) ratio (p=0.024, 95% CI: 0.007 0.075) when compared to pain-free patients. In turn, the \( \frac{tCho}{mI} \) ratio was lower in pain-free SCI patients when compared to healthy controls (p=0.003, 95% CI: -0.069 -0.016).

In SCI patients with NP, a higher \( \frac{tCho}{mI} \) ratio was positively associated with a larger cross-sectional spinal cord area (p=0.006, r=0.714, 95% CI: 0.403 1.0236, n=13, Fig 3B) and a higher pin-prick score (p=0.032, r=0.574, 95% CI: 0.222 0.927, n=14, Fig 3C), but not with NP intensity (p=0.406, r=-0.241, 95% CI: -0.819 0.336, n=14). In pain-free SCI patients, the \( \frac{tCho}{mI} \) ratio was not related to cross-sectional spinal cord area (p=0.881, r=0.055, 95% CI: -0.709 0.818, n=10) or pin-prick score (p=0.676, r=0.152, 95% CI: -0.637 0.940, n=10).

To assess the effect of lesion level, we found that the \( \frac{tCho}{mI} \) ratio was not different between paraplegic and tetraplegic patients with NP (p=0.138, 95% CI: -0.021 0.111, Fig 3D) and paraplegic and tetraplegic pain-free patients (p=0.835, 95% CI: -0.023
Within paraplegic patients, the $t\text{Cho}/mI$ ratio was elevated in patients with NP when compared to pain-free paraplegics ($p=0.022$, 95% CI: 0.013 0.126), but this effect was not seen within the tetraplegic patient group ($p=0.421$, 95% CI: -0.052 0.090).

**Changes of total N-acetylaspartate to myo-inositol**

We first confirmed that there was a difference in $t\text{NAA}/mI$ levels between groups ($p=0.023$) as previously reported for SCI patients and healthy controls in a subset of this population$^9$. SCI patients with NP had a similar $t\text{NAA}/mI$ ratio ($p=0.396$, 95% CI: -0.112 0.258, Fig 4A) as pain-free patients which did also not differ from healthy controls ($p=0.126$, 95% CI: -0.296 0.045). In pain-free patients, $t\text{NAA}/mI$ levels ($p=0.006$, 95% CI: -0.378 -0.071) were lower when compared to healthy controls. Lower levels of $t\text{NAA}/mI$ were associated with greater decrease of cross-sectional spinal cord area in pain-free SCI patients ($p=0.022$, $r=0.709$, 95% CI: 0.252 1.166, $n=10$, Fig 4B) while no association was evident in patients with NP ($p=0.128$, $r=0.445$, 95% CI: -0.028 0.918, $n=13$). Pin-prick score and $t\text{NAA}/mI$ ratio were neither associated in patients with NP ($p=0.405$, $r=0.242$, 95% CI: -0.325 0.809, $n=14$, Fig 4C) nor in pain-free patients ($p=0.117$, $r=0.527$, 95% CI: 0.053 1.002, $n=10$). There was no association between $t\text{NAA}/mI$ levels and NP intensity in patients with NP ($p=0.783$, $r=-0.081$, 95% CI: -0.704 0.541, $n=14$).

There was no difference of the $t\text{NAA}/mI$ ratio between paraplegics with NP and tetraplegics with NP ($p=0.747$, 95% CI: -0.195 0.426, Fig 4D). In pain-free paraplegics, the $t\text{NAA}/mI$ ratio was higher compared to pain-free tetraplegics ($p=0.022$, 95% CI: 0.038 0.390). The $t\text{NAA}/mI$ ratio was not different between paraplegic NP and pain-free patients ($p=1.000$, 95% CI: -0.335 0.426) and neither
was it between tetraplegic NP and pain-free patients (p=0.164, 95% CI: -0.112 to 0.321).

Discussion
This study shows metabolites of neuroinflammation and neurodegeneration and how their levels relate to NP in the spinal cord at cervical level C2/3 by means of non-invasive MRS. In particular, the ratio of tCho (i.e. a marker for an intact myelin and cell membrane turnover) over mI (i.e. a marker of activated glial cells) was increased in chronic SCI patients with NP, but not in pain-free patients. This was distinct from markers of neurodegeneration (i.e. tNAA) that related to cord atrophy but not to NP. This study identified tCho/mI levels as a potential sensitive metabolite biomarker of NP in the injured cervical cord.

Metabolite level changes in the atrophied cervical cord
Irrespective of NP, lower ratios of tNAA/mI and tCho/mI were evident in the atrophied cervical spinal cord of SCI patients when compared to healthy controls. This likely reflects ongoing neurodegeneration and activation of glial cells in the injured spinal cord, especially as the reduction of the metabolic ratios was greater in tetraplegic as compared to paraplegic patients.

Aberrant activity of spinothalamic neurons in the spinal dorsal horn is believed to be a key player in the origin of NP, which manifests as an abnormal sensation of tingling and pricking (i.e. paresthesia), painful sensations induced by non-noxious stimuli (i.e. allodynia), and/or increased or decreased responses to painful stimuli (i.e. hyper- or hypoalgesia). In the spinal cord of SCI patients with NP, this study shows higher tCho/mI ratios than in pain-free patients. Interestingly, in SCI patients with NP the concentration of tCho/mI was in the range of healthy controls. Higher tCho/mI
ratios might relate to enhanced pain transmission in the spinal cord possibly due to an interplay of hyperactive spinothalamic neurons\textsuperscript{35, 37} and inflammatory-induced glial activation\textsuperscript{37}. At the cellular level, this would relate to an increased membrane and myelin turnover (e.g. higher $t\text{Cho}$)\textsuperscript{11} of spinothalamic neurons or activated glial cells (e.g. higher $t\text{Cho}$ and higher $ml$)\textsuperscript{11, 38, 39}. Moreover, $ml$ might be decreased and $t\text{Cho}/ml$ levels therefore increased due to less myelin breakdown in SCI patients with NP\textsuperscript{40}. However, we are unable to disentangle the exact processes at the molecular level, as both $t\text{Cho}$ and/or $ml$ could drive the ratio differences. Nevertheless, we are confident that elevated $t\text{Cho}$ is significantly contributing to the ratio differences, as Widerström-Noga et al.\textsuperscript{15, 17} showed that $t\text{Cho}$ was specifically elevated in distinct brain regions of the pain network (i.e. thalamus and anterior cingulate cortex (ACC)) of SCI patients with NP. Interestingly, these regions, together with the prefrontal cortex (PFC), sensorimotor cortex, and spinal cord, showed NP associated bidirectional volume changes\textsuperscript{41, 42}.

To address the question whether the metabolite level changes relate to NP and/or lesion level, we performed a sub-group analysis between paraplegic and tetraplegic patients. No lesion level-dependent difference in $t\text{Cho}/ml$ levels was evident. However, $t\text{NAA}/ml$ levels were lower in tetraplegic compared to paraplegic pain-free patients which is in agreement with our previous report\textsuperscript{9}, indicating that the magnitude of neurodegenerative processes is lesion level-dependent. This finding is in line with a preclinical study reporting higher $t\text{NAA}$ levels in a rabbit model of SCI with a light compared to a severe injury\textsuperscript{43}. 
Relationship between metabolite level changes, spinal cord atrophy, and pain perception

As recently reported in a subset of this population\textsuperscript{9}, SCI patients showed clinicopathological relationships. However, previous analysis was performed irrespective of NP. In this study, SCI patients with NP showed a threeway relationship between elevated $tCho/ml$ ratios, less cord atrophy, and more preserved pain sensation (e.g. better pin-prick score). This suggests that SCI patients with NP have less degenerated spinal pathways (i.e. less cord atrophy and higher pin-prick scores) and a greater metabolite turnover due to enhanced glial activation and proliferation\textsuperscript{38, 39}. It is thus well imaginable that SCI patients suffering from a secondary complication (i.e. NP), when compared to pain-free SCI patients, show a higher potential for greater functional recovery due to the spared but somewhat disturbed tract and circuit function which is reflected by higher cervical cord $tCho/ml$ levels. Similarly to our results, Grabher et al.\textsuperscript{44} showed an association between better recovery of pin-prick score at 12 months and less cord atrophy immediately after the injury in SCI patients. In contrast in pain-free SCI patients, $tCho/ml$ levels were not associated with cord atrophy or pin-prick sensation. However, a lower $tNAA/ml$ ratio was associated with greater cord atrophy, indicating processes of neurodegeneration. The threeway relationship between metabolite levels, cord atrophy, and function speaks to the potential of levels of $tCho/ml$ as a metabolite marker of inflammatory-induced glial activation and aberrant activity of spinothalamic neurons in NP states.

This study has limitations. First of all, the metabolites measured are presented as ratios\textsuperscript{9} to other metabolites and not as absolute values, unlike MRS values in the brain\textsuperscript{8}. This is due to the current lack of a reliable water reference signal in the spinal cord due to the pulsating surrounding CSF (see Supplementary Figure 1 in\textsuperscript{9}). At
present, it is therefore not possible to draw conclusions on single metabolites and their absolute values in spinal MRS acquisitions. In addition, ratios do not allow making inferences about the directionality of the metabolite level changes. Moreover, the physiological roles of the metabolites are manifold and still not completely known and proven yet. This impedes a reliable discrimination of neuroinflammatory and neurodegenerative processes from other mechanisms. In future, we plan to look at spectroscopic data of specific pain areas within the brain and compare the absolute metabolite levels to the ratios reported in the cervical cord. This could help us to better understand and identify which central nervous system regions and underlying mechanisms contribute to the development of NP. Second, in spinal MRS the spectroscopic voxel covers the entire cord and thus cannot measure individual tracts. We therefore obtained metabolite signals derived from the grey and white matter, simultaneously. Future studies would benefit from smaller, ideally tract specific voxels. Moreover, a more detailed pain assessments including body pain drawings with the extent, intensity, and quality of the perceived pain would enable a better characterization between metabolite turnover and the presence of NP. Third, our sample size was rather small. Given the fact that we were using MRS markers, we were not able to assess the extent of measurement error for the outcome measures. However, this does not invalidate our presented group comparisons and significant findings: it is possible that, in the future, variability due to measurement error may be reduced, in which case required sample sizes would be smaller, reflecting the reduced measurement noise. Finally, gender was not equally represented in our patient cohort. However, the male to female patients’ ratio in this study closely reflects the general SCI population with a ratio of 4:1. Furthermore, healthy controls were age and sex matched.
This study identifies levels of $tCho/ml$, a marker of neuroinflammatory processes, as a discriminator of SCI patients with NP from pain-free SCI patients. Using spinal MRS, spinal cord atrophy assessments, and a clinical measure of pain sensation we identified clinicopathological associations. Thus, $tCho/ml$ levels are a promising metabolite biomarker of neuroinflammation in the context of NP post-SCI. Cervical cord MRS holds potential to be used in clinical trials for patient stratification, therapy monitoring, and outcome prediction.

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## Appendix 1: Authors

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Contribution</th>
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<tbody>
<tr>
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<td>Anke Henning, PhD</td>
<td>Institute for Biomedical Engineering, Zurich</td>
<td>Designed and conceptualized the study; revised the manuscript for intellectual content</td>
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<tr>
<td>Patrick Freund, MD</td>
<td>Balgrist University Hospital, Zurich</td>
<td>Supervised the study; revised the manuscript for intellectual content</td>
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References


Figure legends

Figure 1: Representative planning images of spectroscopic voxel placement and metabolite spectra.

MR spectroscopic voxel of interest and representative spectra including the fitting (red lines) and original signal (gray lines) are shown for a healthy control subject, a pain-free spinal cord injury (SCI) patient, and a SCI patient with neuropathic pain (NP). ppm = parts per million.
Figure 2: Overview of metabolites involved in spinal cord injury-induced processes.

Figure 3: Group comparison of tCho/mI levels and correlation with spinal cord atrophy and clinical outcome measure.

(A+D) Group differences of the ratio of total choline-containing compounds to myo-inositol (tCho/mI) are shown for (A) healthy controls (HC, indicated in gray), pain-free spinal cord injury (SCI) patients (indicated in blue), and SCI patients with neuropathic pain (NP, indicated in red) and (D) paraplegic and tetraplegic spinal cord injury (SCI) patients of the pain-free (indicated in blue) and NP (indicated in red) group separately. (B+C) Rank correlation graphs showing the associations of tCho/mI levels with cross-sectional spinal cord area at spinal level C2/3 (B) and pin-prick score (C) for all SCI patients. Pain-free SCI patients are indicated by blue dots, SCI patients with NP by red dots. Uncorrected P values are reported for significant differences. ns = not significant.
Figure 4: Group comparison of $tNAA/mI$ levels and correlation with spinal cord atrophy and clinical outcome measure.

(A+D) Group differences of the ratio of total N-acetylaspartate to myo-inositol ($tNAA/mI$) are shown for (A) healthy controls (HC, indicated in gray), pain-free spinal cord injury (SCI) patients (indicated in blue), and SCI patients with neuropathic pain (NP, indicated in red) and (D) paraplegic and tetraplegic spinal cord injury (SCI) patients of the pain-free (indicated in blue) and NP (indicated in red) group separately. (B+C) Rank correlation graph showing the association of $tNAA/mI$ levels with cross-sectional spinal cord area at spinal level C2/3 (B) and pin-prick score (C) for all SCI patients. Pain-free SCI patients are indicated by blue dots, SCI patients with NP by red dots. Uncorrected P values are reported for significant differences. ns = not significant.
Tables

Table 1: Epidemiological and clinical data for all spinal cord injury patients.

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AIS = American Spinal Injury Association Impairment Scale; NP = neuropathic pain.
Metabolites of neuroinflammation relate to neuropathic pain after spinal cord injury
Dario Pfyffer, Patrik O. Wyss, Eveline Huber, et al.
Neurology published online June 26, 2020
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