Cerebrospinal fluid markers of glial and axonal injury are altered and correlate with symptoms and outcome in patients with cervical spondylotic myelopathy

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Running Head: Cerebrospinal fluid markers of glial and axonal injury in cervical spondylotic myelopathy **ABSTRACT**

Objective: Degenerative cervical spondylotic myelopathy (CSM) is a major cause of spinal cord dysfunction with unpredictable prognosis. Biomarkers reflecting pathophysiological processes in CSM have been insufficiently investigated. We hypothesized that preoperative cerebrospinal fluid (CSF) biomarkers are altered in CSM patients and correlate with neurological status and outcome.

Methods: CSF biomarkers from CSM patients and controls were analyzed by immunoassays. Spinal cord changes were evaluated with magnetic resonance imaging (MRI). The American Spinal Cord Injury Association Impairment Scale (AIS), the Japanese Orthopaedic Association Cervical Myelopathy Evaluation Questionnaire (JOACMEQ) and EuroQol 5-dimensions (EQ-5D) questionnaires were assessed prior to and at three months post-surgery. A p value below 0.05 was considered statistically significant.

Results: Twenty consecutive CSM patients aged 67.7 ±13 years and 63 controls aged 65.2 ± 14.5 years were included ($p>0.05$). In CSM subjects, CSF neurofilament light (NF-L) and GFAP concentrations were higher ($p<0.05$), whereas fatty acid-binding protein 3 (FABP3), soluble amyloid precursor proteins (sAPPα and sAPPβ) and amyloid β peptide (Aβ38, Aβ40 and Aβ42) concentrations were lower compared to controls ($p<0.05$). Amyloid β peptide levels correlated positively with symptom duration. Age correlated positively with glial fibrillary acidic protein (GFAP) and chitinase 3-like 1 (YKL-40) levels ($p<0.05$). The preoperative JOACMEQ lower extremity function and CSF NF-L levels correlated positively, and the JOACMEQ bladder correlated negatively with sAPPα and sAPPβ ($p<0.05$). CSF NF-L and FABP3 levels were higher in patients with improved outcome (EQ-VAS difference >20).

Conclusions: CSF biomarkers of glial and axonal damage, inflammation and synaptic changes are altered in symptomatic CSM patients indicating that axonal injury, astroglial activation and Aβ dysmetabolism may be present in these patients. These findings reflect CSM pathophysiology and may aid in prognostication. However, future studies including larger patient cohorts, postoperative biomarker data and imaging and longer follow-up times are required to validate the present findings.

KEY WORDS: Cervical spondylotic myelopathy; biomarkers; glial injury; axonal damage; prognosis

Degenerative cervical spondylotic myelopathy (CSM) is a leading cause of spinal cord dysfunction.[4,17] Treatment of CSM is surgical with the primary goal to arrest progressive neurologic deterioration, although a degree of clinical improvement is commonly observed.[11,19] Prognosis for neurological recovery following surgery for CSM is dependent on many factors including preoperative neurological status, age, duration of symptoms, as well as preoperative magnetic resonance imaging (MRI) findings and neurophysiological changes.[4,19,27,41] Furthermore, surgical treatment has been associated with better neurological recovery in CSM patients with a symptom duration of less than 12 months (PMID: 12493108). Only few reports investigating biomarkers in CSM exist.[26,38,39,42] In patients operated for CSM, cerebrospinal fluid (CSF) pNF-H levels correlated positively with outcome, in particular in patients with acute preoperative clinical worsening.[38,39] In the present study, we hypothesized that in symptomatic patients with CSM: 1. The levels of biomarkers in CSF for axonal injury, neurodegeneration and neuroinflammation are altered and, 2. The biomarker levels in CSF are associated with preoperative neurological status, MRI findings and clinical outcome following surgery.

Methods

Patients and setting

The study was approved by the regional ethics committees of Uppsala, Sweden and Gothenburg, Sweden and was conducted in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2008. All subjects were prospectively enrolled and signed a written informed consent form prior to inclusion in the study.

Patients with progressive clinical symptoms and radiological findings suggestive of CSM admitted for neurosurgical treatment at the Department of Neurosurgery, Uppsala University Hospital, Uppsala, Sweden were recruited. All patients underwent a neurological examination and were evaluated using the American Spinal Injury Association (ASIA) Impairment Scale (AIS).[20]

Control CSF samples were obtained from patients without any known neurological disorder who underwent spinal anesthesia for elective orthopedic surgical procedures at the Sahlgrenska University Hospital, Mölndal, Sweden.

Radiology

Prior to surgery, a routine magnetic resonance imaging (MRI) of the cervical spine was performed using T1- and T2-weighted images that included axial and sagittal projections. The degree of spinal cord compression and the number of affected levels were analyzed by an investigator (PPT) blinded to the biomarker levels and clinical outcome of each patient. Hypointensity signals on T1-weighted images and hyperintensity signals on T2-weighted images were analyzed, as was the rostrocaudal length of high signal intensity on the T2-weighted sequence.[7,27,45]

Surgery

Patients with progressive CSM were offered surgical treatment, to which all included patients consented. Patient-related factors, spinal configuration and extent of compression decided the surgical approach. For posterior approaches, a laminectomy with or without posterior fusion was chosen. Routine anterior cervical discectomy was used for ≤ three-level disc pathology using fusion cages with or without a concomitant cervical plating. A corpectomy, using an iliac crest bone graft or an adjustable cage was also a surgical alternative.

Outcome measures

The Japanese Orthopaedic Association Cervical Myelopathy Evaluation Questionnaire (JOACMEQ) and EuroQol 5-dimensions (EQ-5D) were filled out on the day prior to surgery and at follow-up three months postoperatively. The questions of JOACMEQ are divided into five dimensions reflecting cervical spine function, upper extremity function, lower extremity

function, bladder function and quality of life.[14,40]

The EQ-5D instrument is a standardized measure of health status developed by the EuroQol Group to provide a simple, generic measure of health for clinical and economic appraisal. The dimensions of EQ-5D are mobility, self-care, usual activities, pain/discomfort and anxiety/depression. The dimensions are rated by the responder as 1=no problem, 2=some problems or 3=extreme problems. This rating is accompanied by an EQ-5D Visual Analog Scale (VAS) value between 0 and 100 (self-rated scale), evaluating the Quality of Life-situation, where 0 is "worst imaginable health state" and 100 is "best imaginable health state" [10] where a minimum important difference of at least 8 has been found significant.[31]

All surgical and systemic complications were noted. At three months following surgery, a researcher (UH) assessed all postoperative JOACMEQ and EQ-5D by phone interviews.

Biomarker analysis

Cerebrospinal fluid (CSF) for biomarker analyses was obtained the day prior to surgery using routine lumbar puncture. CSF samples were collected and centrifuged at 3600 rpm for 10 min. The CSF was then aliquoted into 1mL samples and stored at -72°C until analysis. The samples were sent to the Clinical Neurochemistry Laboratory, Department of Psychiatry and Neurochemistry, University of Gothenburg, Sweden for biomarker analyses. The following biomarkers with known involvement in neurodegeneration or acute central nervous system (CNS) injury were evaluated: glial fibrillary acidic protein (GFAP, a marker of astrocytic activation), neurofilament light (NF-L, a marker of axonal injury), tau (another marker of axonal injury), heart-type fatty acid-binding protein (FABP3, a neuronal injury marker), chitinase 3-like 1 (YKL-40, a marker of astrocytic activation), soluble amyloid precursor protein α (sAPPα, a marker of non-amyloidogenic APP processing), soluble amyloid precursor protein β (sAPPβ, a marker of amyloidogenic APP processing), amyloid β 38, 40 and 42 (Aβ38, Aβ40 and Aβ42, markers of amyloidogenic APP processing, with Aβ42 specifically related to Alzheimer-associated plaque pathology). GFAP concentration was measured using an in house ELISA, as previously described.[34] NF-L concentration was measured using a commercially available kit

(NF-Light, Uman Diagnostics, Umeå, Sweden), according to the kit insert. FABP3 concentration was measured using an immunoassay with electrochemiluminescence detection (MSD® Human FABP3 kit, Meso Scale Discovery, Rockville, MD). YKL-40 concentration was measured using a commercial ELISA (R&D Systems, Minneapolis, MN). sAPPα and sAPPβ were measured using an sAPP Duplex kit (Meso Scale Discovery, Rockville, MD). Aβ38, Aβ40 and Aβ42 concentrations were measured using an Abeta Triplex kit (Meso Scale Discovery, Rockville, MD). All assays were performed on one occasion using one batch of reagents by board-certified laboratory technicians who were blinded to clinical data. Intra-assay coefficients of variation were below 10%.

Statistical analysis

Normality of data was tested with the Kolmogorov – Smirnov test. Normally distributed data are presented as mean and standard deviation and non-normally distributed data as medians and interquartile range. Continuous variables were evaluated for statistical significance using the Student's t-test, Wilcoxon's rank sum or Mann Whitney U, as appropriate. A univariate analysis of correlation between age, gender, number of affected spine levels, biomarker levels, radiological findings, duration of symptoms and outcome from JOACMEQ, EQ-5D and EQ-VAS was calculated using the Spearman Rank Correlation. Due to the relatively small number of CSM patients, a multivariate analysis was not performed. The duration of symptoms (≤1 year vs. >1 year) and EQ-VAS difference (<10 vs. >10 and <20 vs. >20) was dichotomized as dependent variables with the biomarker level as the independent variable.[31] In selective occasions, the area under the receiver operating characteristic (AUROC) curve was also calculated.

Data was analyzed using the statistical software SPSS version 22 (IBM, New York, NY, USA) and GraphPad Prism 7 (San Diego, California, USA). Statistical significance was set at $p < 0.05$.

Results

Patients and controls

Twenty CSM patients, 12 men and 8 women aged 32-89 (mean 67.7 ±13) years, were included. Sixty three controls, 32 men and 31 women aged 22-95 (mean 65.1±14,5) years were included with no difference in age compared to CSM patients (p=0.50). The self-reported symptom duration related to CSM was 12 months or less in 11 patients and in 9 patients longer than 12 months (median 10.0 [range 1-132] months). Patient characteristics are shown in Table 1.

Fourteen patients were in good preoperative health, while two had previous treatment for malignancy, two had diabetes mellitus, two had rheumatoid arthritis of whom one also had minor sequelae from a previous stroke. All patients were AIS D. Control patients (n=63; 35 females, 28 males) had an average age of 65.2 ± 14.5 years (range 22-95).

Radiology

An MRI was performed on all patients prior to surgery. A low signal on T1-weighted sequence was found in 8 patients. Signal changes on T2 weighted images were present in 14/20 patients (mean length of signal intensity 8.2 ± 2.9 mm, range 2.7-12.9 mm; Table 1). Eight patients had signal changes on both T1- and T2- weighted images and 5 patients had no signal changes on either T1- or T2-weighted images. In most patients (13/20), myelopathy symptoms resulted from disc herniation (Table 1).

Surgical data

Most patients (n=15) were subjected to anterior cervical discectomy and fusion (ACDF). In four cases, a laminectomy was performed. One patient was operated with a C4-5 anterior corpectomy and fusion. Ten patients had surgery at one level, nine at two levels and one at three levels and one patient had a previous ACDF surgery. The patient who underwent

corpectomy required revision surgery two months postoperatively due to instrumentation failure (Table 1).

Biomarker analysis

NF-L, GFAP, Tau, FABP, and YKL-40 levels in CSF

The NF-L and GFAP biomarker levels in CSF were significantly higher in the CSM group compared to controls (p=0.0003 and p=0.0002, respectively; Table 2 and Fig. 1).

FABP3 levels in CSF were significantly lower in patients with CSM compared to controls (p<0.0001; Fig. 1), while tau and YKL-40 levels were similar in CSM patients and controls (p=0.058 and p=0.105, respectively; Table 2, Fig.1 and Fig. 2).

Amyloid breakdown products in CSF

sAPPα, sAPPβ, Aβ138, Aβ40 and Aβ42 levels in CSF were all significantly lower in the CSM group compared to controls (p<0.01; Fig. 2).

Subjects with myelopathy symptoms ≤1 year had significantly higher NF-L levels in CSF compared to those with symptom duration >1y with an AUROC of 0.888 (p<0.003, 95% CI: 0.715-1.063; Fig. 3) (NF-L ≤1 year). No difference was found in any of the other evaluated biomarkers (p>0.05). Aβ38, Aβ40 and Aβ42 levels in CSF of patients with CSM correlated positively with the duration of symptoms (p<0.05; Fig. 2) as in those with longer symptom duration, higher amyloid levels were observed.

Correlation of biomarker levels with MRI findings

Signal changes on T1 and T2 weighted images correlated with biomarker levels. A significant correlation between T1 weighted signal changes (low signal) and NF-L levels was observed (AUROC 0.817; p=0.012, 95% CI: 0.623-1.012; Fig. 3). No association between the T1 and T2

signal changes and the levels of any of the other biomarkers was found ($p>0.05$). Similarly, the degree of signal change on T2 sequence did not correlate with the levels of any biomarker ($p<0.05$).

Clinical Outcome and correlation with biomarkers

Assessment of outcome data was performed for 19 patients as one male patient (# 3) was lost to follow-up. The JOACMEQ improvements after surgery in all dimensions are presented in Figure 4. In all dimensions except for bladder function, surgery resulted in significant clinical improvement ($p<0.05$, Fig. 4). There was an overall improvement for the whole group (n=19), measured with the EQ-5D index from before 0.36 (IQR: 0.07-0.69) to after surgery 0.69 (IQR: 0.52-0.73), $p<0.05$. EQ-VAS (self-assessment of health status) significantly improved from 40.5 ± 19.1 to 64.5±16.1 ($p<0.05$).

Correlation of biomarker levels with clinical parameters

Age correlated positively with GFAP and YKL-40 levels ($p<0.05$). No association between gender and number of affected spine levels with any of biomarker concentrations was found ($p>0.05$, Fig. 5).

The preoperative JOACMEQ lower extremity function correlated negatively with CSF NF-L levels, and between JOACMEQ bladder, sAPPα (and sAPPβ (negative correlation) ($p<0.05$; Fig. 4). There was no correlation between biomarker levels and 3-month outcome according to any of the JOACMEQ dimensions ($p>0.05$).

Subjects with EQ-VAS difference > 20 at 3 months had significantly higher levels of NF-L and significantly lower levels of FABP3 compared to those with EQ-VAS difference < 20 with an AUROC of 0.784 and 0.818, respectively ($p=0.04$, 95% CI: 0.570-0.997 and $p=0.02$, 95% CI: 0.621-1.015; Fig. 6). No correlation between CSF biomarker levels and 3-month outcome according to any of EQ-5D dimensions and an EQ-VAS difference of >10 was found ($p>0.05$).

Discussion

In the present report, we found that in patients with CSM, CSF levels of NF-L and GFAP were higher while amyloid breakdown products were lower compared to controls. In addition, the amyloid peptides Aβ38, Aβ40 and Aβ42 all correlated positively with the duration of symptoms. The preoperative lower extremity function correlated positively with CSF NF-L levels, while there was a negative correlation between bladder function and sAPPα and sAPPβ. Symptom duration of less than 1 year was associated with better outcome. In addition, CSF NF-L and FABP3 levels were higher in patients with an improved clinical outcome. The present data suggest that CSF biomarkers can reflect the ongoing pathophysiology of spinal cord compression and damage and may provide prognostic information on surgical outcome.

When the spinal cord is compressed, chemical factors reflecting structural nervous tissue injury may be released into the CSF and blood. These biomarkers have mainly been investigated in acute spinal cord injury (SCI) where, specifically, neurofilament (NF-L and NF-H) and tau for axonal damage, neuron-specific enolase (NSE) for neuronal damage, myelin basic protein (MBR) for demyelination, S100B and GFAP for astrocytic injury have been investigated.[1,9,22-24] These markers, in combination with a cytokine response, have shown to reflect injury severity and outcome following acute SCI.[9,24] However, few previous reports evaluating a limited number of biomarkers have used blood or CSF in the prognostication and preoperative evaluation of CSM.[38,39]

The pNF-H (heavy chain) subunit is a main component of the axonal cytoskeleton (16176808) that has previously been associated with injury severity in acute SCI (29291173). pNF-H was also increased in CSF of patients with spinal cord tethering and neurological deterioration, suggesting ongoing axonal degeneration and supporting its use as a potential biomarker for chronic SCI (32588294). In acutely deteriorating CSM patients, pNF-H levels were increased and correlated with improved recovery after surgery. [31,38,39] Of them, Takahashi *et al*. found significantly higher levels of CSF pNF-H in patients with acutely worsening myelopathy symptoms in comparison to patients with chronic compression myelopathy or lumbar canal

stenosis . The JOA score improved more in acute myelopathy than in patients with chronic symptoms, a finding attributed to compensatory axonal plasticity (PMID 29557051).

The rationale for using NF-L was based on its increasing importance in other CNS injuries, notably traumatic brain injury and sports-related concussion. It is a rather sensitive and specific biomarker for the detection of injured large caliber, myelinated axons which is rapidly released following axonal injury at prolonged post-injury time points (29209266) [3,44]. In the current cohort, CSF NF-L levels were elevated compared to controls. NF-L levels were higher in patients with symptom duration <1 year. Interestingly, NF-L levels correlated significantly with low T1 signal on MRI but not with T2 signal, possibly indicating a more severe tissue injury in these patients (PMID: 1895114).[5,36] Therefore, it can be hypothesized that NF-L might serve more as a marker of recent axonal damage rather than as a marker of chronic axonal damage. Of note, tau, also a suggested marker of axonal injury in traumatic brain injury, did not significantly differ compared to controls, possibly implying different sensitivity for spinal cord compression for these two biomarkers.

CSF GFAP levels were also elevated in patients with CSM compared to controls. In a previous study, elevated levels of GFAP were found in serum in the first 24 hours following SCI, which then decreased in the coming days.[1] Age correlated positively with GFAP and YKL-40 concentrations possibly implying altered astroglial activation in older individuals. GFAP levels also correlated with injury severity in both moderate and severe SCI, as well as in traumatic brain injury.[1,16,24,35] GFAP levels have not been previously reported in CSM patients and while CSF GFAP levels were elevated, they were not linked to symptom duration. Although the role of GFAP in the nervous system is complex, it may suppress neuronal proliferation and neurite extension in the mature brain and can play a role in the formation of a barrier restricting the extent of injury. Since our data and others [6] suggest that astroglial activation occurs also in CSM, GFAP release may reflect the tissue injury induced by the spinal cord compression and be an important factor in the recovery process following CSM surgery.

We observed low levels of amyloid-β breakdown products in CSF. Following traumatic brain injury, Aβ species are detected in CSF.[13,29,43] However, their levels have been highly variable in the literature, an observation possibly related to the methods used for their detection and the

underlying pathology. There is evidence to support that, in the acute phase of CNS injury, Aβ levels rise while they decrease later in the course of the disease, which might explain the low levels found in the present study.[29,32] As an example, in severe TBI patients, low Aβ levels were found in CSF samples analyzed by ultrasensitive digital immunoassay.[29,32] Decreased Aβ40 and Aβ42 were recently reported in subjects with repetitive concussive brain injury.[35] Amyloid peptide species in the present sample of myelopathy patients can be indicative of amyloid aggregation and plaque formation as well as axonal damage, decreased synaptic activity and secretion or altered clearance of Aβ peptides from the nervous system.[29,35] . While spinal cord compression is segmental, a widespread axonal dysfunction is also possible since further, axonal compression injury may cause a disturbed axonal transport with accumulation of proteins such as APP.[8,15] which in turn would result in lowering of synaptic release of its breakdown products sAPP and Aβ to the extracellular space and CSF. However, whether altered synaptic activity is the explanation for the correlation of CSF Aβ levels and JOACMEQ dimensions and duration of symptoms is something which, at this point, cannot be clearly stated and should be explored in future studies.

FABP3 is a cytoplasmic protein released from cardiac myocytes following an ischemic event, which in humans is encoded by the FABP3 gene.[21,33] It is a sensitive biomarker for myocardial infarction and following pulmonary embolism.[18] A potential role as a diagnostic marker in the differential diagnosis of dementias was also suggested.[21,28] Further, in patients with mild TBI, high levels of H-FABP were found in patients with positive computed tomography (CT) scan findings.[25] Our study is the first to show that it may also play a role in the compressed spinal cord, although at present, the mechanisms underlying the low CSF levels of FABP3 compared to controls are unknown.

While there was a degree of cervical spinal stenosis in all CSM patients, MRI signal changes in the spinal cord were not uniformly present since the diagnosis of CSM is predominantly clinical. However, the presence of low signal on T1-weighted images and high signal on T2-weighted images has been suggested to indicate a worse clinical status and neurological improvement after decompressive surgery.[2,12,30,37,44] In the present cohort, a significant correlation of T1 signal with NFL was found, but the MRI findings were not associated with

clinical outcome. It should be mentioned though that high T2 signal intensity cannot always be indicative for spondylotic compression myelopathy since other pathologies can also induce signal changes on T2 sequence.[30,37]

This study has some limitations. The patient material of 20 patients showed some heterogeneity in terms of clinical condition, duration of symptoms and co-morbidities. Thus, this cohort may not fully reflect the broad clinical and radiological spectrum of CSM and the clinical significance of altered biomarker levels is still somewhat inconclusive. Especially in severe CSM cases, lumbar CSF may have been stagnant and CSF samples obtained by lumbar puncture may not adequately reflect the release of biomarkers from a compressed and injured spinal cord. Furthermore, the follow-up time was rather short, follow-up data was obtained through telephone interview and additional clinical change may have occurred at later stages. In future studies, comparisons of preoperative to postoperative CSF biomarker levels are warranted. A postoperative MRI scan was not routinely done in our unit. Therefore, possible alterations of signal changes in the spinal cord could not be assessed. These limitations should all be addressed in coming studies. However, the present report used a panel of biomarkers and found distinct changes in CSM patients which likely represent important pathophysiological events and may have a future role in the clinical management and prognostication of CSM.

In the present work, the follow-up was performed at three months post-surgery. This time point was selected based on the clinical routine in our department and was also specified in the ethical permission. In spinal cord injury, while a degree of clinical improvement could occur beyond three months most clinical recovery occurs in the first three months post-injury (PMID: 19831845, 17179973). Furthermore, in cervical spondylotic myelopathy, numerous previous studies have shown that neurological recovery after surgical decompression is fast during the initial post-surgery months after which it soon reaches a plateau (PMID: 24777670, 30685954, 17235616, 31503050)**.** Thus, the degree of neurological recovery at 3-months post-surgery presumably reflects the long-term outcome, However, this is something that should be validated in future studies

Conclusions

In patients with cervical spondylotic myelopathy, biomarkers of glial and axonal damage in CSF biomarkers are increased, while others are decreased. These findings indicate that axonal injury, astroglial activation and Aβ dysmetabolism may be present in CSM patients. Our data imply a clinical importance of these biomarker, which may help better explain the progression of CSM and possibly response to surgical decompression. However, the pathophysiology of CSM remains largely unclear since future studies including larger patient cohorts, postoperative biomarker data and imaging and longer follow-up times are required to validate the present findings.

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PP. Tsitsopoulos, U.Holmström and N. Marklund report no disclosures related to this work. K. Blennow has served as a consultant on advisory boards for Eli Lilly, Fujirebio Europe, IBL International, Novartis, and Roche Diagnostics. H. Zetterberg has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. K. Blennow has served as a consultant or at advisory boards for Abcam, Axon, Biogen, Lilly, MagQu, Novartis and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg.

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Ethical considerations

The prospective study was approved by the local ethics committee (Decision Number 400/2012). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the with the ethical standards of the Helsinki Declaration of 1975, as revised in 2008. All recruited patients signed a written informed consent form prior to study inclusion.

Author contributions

Conception and design: Holmström, Marklund. Acquisition of data: All authors. Analysis and interpretation of data: All authors. Drafting the article: Holmström, Marklund, Tsitsopoulos. Critical revising the article: All authors. Reviewed and approved the final version of the article: All authors. Administrative, technical, or material support: Blennow, Marklund, Zetterberg. Statistical analysis: Holmström, Marklund, Tsitsopoulos. Obtained funding: Blennow, Marklund, Zetterberg. Study supervision: Marklund.

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Figure Legends

FIG 1. (A-D) Biomarker levels of patients with cervical spondylotic myelopathy (CSM) in cerebrospinal fluid (CSF) compared to controls for NF-L, GFAP, FABP3 and YKL-40 The levels of NF-L and GFAP were higher than controls whereas CSF levels of FABP3 were lower than controls ($p<0.05$). Biomarker values are presented in pg/ml. FABP3=Fatty Acid Binding Protein 3; GFAP= Glial fibrillary acidic protein; NF-L= Neurofilament light; YKL-40= Chitinase-3-like protein 1 (CHI3L1), also known as YKL-40.

FIG 2. (A-C) Amyloid breakdown products and tau levels of patients with cervical spondylotic myelopathy (CSM) in cerebrospinal fluid (CSF) compared to controls. The CSF levels of amyloid peptides Aβ38, Aβ40 and Aβ42 were lower in CSM patients compared to controls ($p<0.05$).

(D-I) Duration of symptoms positively correlated with CSF levels of Aβ38 ($R^2=0.297$), Aβ40 ($R^2=0.261$) and Aβ42 ($R^2=0.270$) ($p<0.05$). The CSF levels of sAPPα and sAPPβ were also decreased in the CSF compared to controls ($p<0.05$) while the CSF Tau levels were similar to controls ($p>0.05$). Biomarker values are presented in pg/ml. Aβ= amyloid-beta, sAPP= soluble Amyloid Precursor Protein.

FIG 3. (A-B) Patients with cervical spondylotic myelopathy (CSM) showing changes on T1 weighted signal changes (low signal, T1+) on preoperative Magnetic Resonance Imaging (MRI) had higher NF-L levels ($p=0.012$) which was associated with a prognostic utility of 0.817 on AUROC.

(C-D) CSM patients with symptom duration ≤ 1 year had higher NFL values in cerebrospinal fluid (CSF) compared to those with symptom duration > 1 year ($p=0.002$), a finding associated with high prognostic value on AUROC (0.888). AUROC= the area under the receiver operating characteristic; NF-L=Neurofilament light

FIG. 4. (A) Changes in median values for the Japanese Orthopaedic Association Cervical Myelopathy Evaluation Questionnaire (JOACMEQ) scores from prior to and at three months after surgery. In all dimensions except for bladder, a significant improvement was observed. One patient was lost to follow-up.

(B-D) NF-L levels in cerebrospinal fluid (CSF) positively correlated with JOACMEQ lower (R^2=0.563) whereas sAPPα (R^2=-0.207) and sAPPβ (R^2=-0.277) levels in CSF inversely correlated with JOACMEQ bladder. sAPP= soluble Amyloid Precursor Protein.

FIG 5. (A-B) In patients with cervical spondylotic myelopathy, age correlated positively with GFAP (r^2=0.284) and YKL-40 (r^2=0.273) levels. GFAP=Glial Fibrillary Acidic Protein; YKL-40=Chitinase 3-like 1.

FIG. 6. (A-D) Patients with cervical spondylotic myelopathy (CSM) and EQ-VAS improvement of ≥ 20 had higher NF-L and FABP3 values in the cerebrospinal fluid (CSF) preoperatively. NFL values had a prognostic utility of 0.784 and FABP3 of 0.818 according to AUROC. The remaining biomarkers did not correlate with EQ-VAS improvement. AUROC= the area under the receiver operating characteristic; FABP3= Fatty Acid Binding Protein 3; NF-L= Neurofilament-light.