Deutsche Wirbelsäulenstiftung

Geschäftsstelle

Parkweg 6

07751 Jena

Abschlussbericht des geförderten Projektes:

„Die sekundären Schädigungsmechanismen der degenerativen zervikalen Myelopathie“

Antragssteller:

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Projekt-Code: 36164

Sehr geehrte Damen und Herren,

zunächst möchten wir uns für die Förderungen durch die Deutsche Wirbelsäulen-Stiftung bedanken. Wir konnten mit dieser Unterstützung unsere Biodatenbank mit Liquor Proben und Serum Proben von Patienten mit degenerativer zervikaler Myelopathie erweitern. Ebenfalls war es uns möglich mit den bereitgestellten Mitteln weitere Messungen von Biomarkern in Bezug auf Inflammation und Angiogenese durchzuführen. Bereits in Vorarbeiten war es uns möglich Veränderungen bestimmter Mediatoren dieser endogenen Reaktion im Liquor von Myelopathie Patienten zu detektieren. Durch die nun bewilligte Förderung waren wir in der Lage diese Untersuchung fortzuführen. Ein positives Ethik Votum der unabhängigen Ethikkommission der Medizinischen Fakultät in Aachen ermöglicht es uns auch postoperativ Liquor Proben zu gewinnen. Dies schafft wissenschaftlich ein Alleinstellungsmerkmal in der molekularen Grundlagenforschung der degenerativen zervikalen Myelopathie.

Bei der Probensammlung, bzw. deren Lagerung hat es leider technische Probleme gegeben. Gesammelte Proben waren nicht ausreichend gekühlt worden (geplante Lagerung bei –80°). Es wurde

von unserer Seite eine Verlängerung bezüglich der Bereitstellung der bewilligten Mittel erbeten. Wir konnten in dem Zeitraum dieser Verlängerung sicherlich nicht unser angestrebtes Ziel der Patientenzahlen und Kontrollen erreichen. Jedoch konnten wir insgesamt 50 Patienten mit Myelopathie einschließen, bei 20 Patienten konnten wir nach 3 Monaten postoperative Proben asservieren. Als Kontroll-Gruppe dienen uns Patienten mit aortalem Aneurysma, hier konnten letztlich 52 Patienten eingeschlossen werden.

Es wurden dann mit den asservierten Proben Messungen durchgeführt. Durch unsere Vorarbeiten (NATURE-SCIENTIFIC REPORTS (2021): Decreased angiogenesis as a possible pathomechanism in cervical degenerative myelopathy) waren zwei Faktoren von besonderem Interesse: Angiopoietin-2 und VEGF-C. Diese Faktoren sollten nun bei den neu eingeschlossenen Patienten und postoperativ gewonnen Proben untersucht werden.

Die Ergebnisse konnten wir bereits in ein Manuskript einfügen. Dieses fügen wir an dieser Stelle in den Abschlussbericht ein:

Angiopoietin-2 and VEGF-C Levels in Cerebrospinal Fluid of Patients with Degenerative Cervical Myelopathy: Pre- and Post-Surgical Analysis and Correlations with Clinical Outcomes.

Abstract

Background

Degenerative cervical myelopathy (DCM) is a major cause of spinal cord injury globally, associated with both primary mechanical injury and secondary inflammatory and degenerative processes. Angiopoietin-2 (Ang2) and Vascular Endothelial Growth Factor C (VEGF-C) are critical in modulating inflammation and vascular function, yet their role in DCM remains insufficiently understood. This study investigates changes in CSF levels of Ang2 and VEGF-C in DCM patients before and after decompressive surgery and explores correlations with clinical outcomes.

Methods

A cohort of 50 DCM patients undergoing decompressive surgery were recruited, with CSF samples obtained preoperatively (DCMpre) and three months postoperatively (DCMpost) from a subset of 20 patients. A control group consisted of 52 neurologically healthy patients undergoing thoracoabdominal aortic aneurysm (TAAA) surgery. Ang2 and VEGF-C levels in CSF were measured using ELISA and magnetic bead assays, respectively. Clinical outcomes were assessed with the modified Japanese Orthopaedic Association (mJOA) score, Neck Disability Index (NDI), and Oswestry Disability Index (ODI), and statistical analyses included ANOVA, paired t-tests, and Spearman correlation.

Results

Ang2 levels were significantly lower in DCM patients (DCMpre: 276 ± 90 pg/mL, DCMpost: 277 ± 65 pg/mL) compared to the control group (463 ± 240 pg/mL, $p < 0.001$). A paired t-test revealed a significant increase in Ang2 levels post-surgery in patients symptomatic for over six months ($p < 0.05$). VEGF-C levels showed no significant differences across groups. Preoperative Ang2 levels correlated moderately with disability indices (NDI: $r = -0.46$, ODI: $r = -0.44$, both $p < 0.001$), but no significant correlations were observed with mJOA scores or VEGF-C levels.

Conclusions

This study highlights reduced CSF Ang2 levels in DCM patients compared to healthy controls, with a post-surgical increase in patients with prolonged symptom duration. While Ang2 correlated with disability indices, neither Ang2 nor VEGF-C were effective biomarkers for DCM symptom severity or recovery. Further studies with larger cohorts are necessary to evaluate their potential roles in disease progression and therapeutic monitoring.

Funding

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Introduction

Degenerative cervical myelopathy (DCM) is a leading cause of spinal cord injury globally and presents a substantial socioeconomic burden, particularly in aging populations in developed countries[1,2].

DCM involves primary damaging mechanisms, including mechanical compression of the spinal cord and ischemia, along with secondary mechanisms, such as neuroinflammation, apoptosis, and progressive ischemia, which exacerbate neuronal degeneration as downstream effects of the primary injury[1].

While the concept of secondary injury in spinal cord pathology was introduced in the early 20th century, no pharmacotherapeutic interventions currently target secondary mechanisms in DCM patients.[3,4]. Early decompressive surgery remains the primary recommended treatment.[4]. One of the challenges in developing pharmacological therapies is the limited understanding of pro-apoptotic, inflammatory, and angiogenetic mechanisms specific to DCM.

Our previous study identified significantly decreased levels of Angiopoietin-2 (Ang2) and Vascular Endothelial Growth Factor C (VEGF-C) in cerebrospinal fluid (CSF) samples of

symptomatic DCM patients at the time of decompressive surgery compared to asymptomatic patients.[5]

Ang2 and VEGF-C are essential in modulating inflammation and vasculogenesis, each affecting vascular stability and immune response mechanisms. Ang2 influences vascular remodeling by binding to the Tie2 receptor on endothelial cells, generally promoting vascular instability under inflammatory conditions.[6]. Interestingly, we found both decreased Ang2 CSF levels along with clear evidence of a blood-brain/spinal cord- barrier disruption in DCM patients[5]. Similarly, VEGF-C is crucial in lymphangiogenesis, promoting immune cell trafficking and edema resolution, with implications in neurodegenerative diseases like Alzheimer's[7,8].

This study aims to investigate CSF Ang2 and VEGF-C concentrations in DCM patients before and three months after decompressive surgery, comparing these levels to a control group of neurologically healthy patients. We hypothesize that (A) Ang2 and VEGF-C levels will change following decompression and (B) these changes may correlate with clinical outcomes, potentially serving as biomarkers for treatment response.

Methods

Symptomatic DCM patients eligible for decompressive surgery, defined as having a modified Japanese Orthopaedic Association (mJOA) Score ≤ 17/18 and cervical spinal cord compression evident on MRI, were recruited from 2015 to 2020. CSF samples were obtained preoperatively from 50 patients (DCMpre) and from 20 patients three months post-surgery (DCMpost). All samples were acquired via lumbar puncture under sterile conditions. The control group consisted of 52 patients undergoing surgery for thoracoabdominal aortic aneurysm (TAAA) who had no neurological symptoms and received lumbar drainage as part of the standard surgical protocol.

Patients with prior spine surgeries were excluded. All recruited individuals gave written consent to take part in this study. The study was conducted in accordance with the 2013 revision of the declaration of Helsinki.

Demographic data, including age, sex, body mass index (BMI), and symptom duration, were recorded. Symptom burden was assessed using standardized questionnaires: mJOA Score, Neck Disability Index (NDI), Oswestry Disability Index (ODI), and a visual analog scale (VAS) for neck and arm pain, both preoperatively and three months postoperatively for the DCMpost group.

Sample acquisition

Pre-surgical CSF samples from DCM patients were collected via lumbar puncture under sterile conditions following general anesthesia but before surgical incision. Postoperative CSF samples were obtained from DCM patients three months post-surgery, with or without local anesthesia. TAAA patient samples were collected directly from the lumbar drain. All samples were stored at -80°C until analysis.

CSF analytics

Ang2 concentrations in CSF were measured using the Human Angiopoietin-2 ELISA Kit (KHC1641, Thermo Fisher Scientific, Waltham, MA, USA) with data acquisition through an Infinite M200 Plate Reader (Tecan, Männedorf, Switzerland). VEGF-C levels were analyzed using the Human Angiogenesis/Growth Factor Magnetic Bead Panel (Milliplex HAGP1MAG-12K-01, Merck, Darmstadt, Germany) on the Luminex MAGPIX system (Diasorin, Saluggia, Italy). Data were processed with ProcartaPlex Analyst 1.0 (Affymetrix eBioscience, Santa Clara, CA, USA). All assays followed manufacturer protocols, and samples were pipetted in triplicate.

Statistical analysis

Data were first assessed for normality. ANOVA followed by Tukey's post hoc test was applied to Ang2 data, with ratio-paired t-tests between DCMpre and DCMpost groups. VEGF-C data were analyzed using the Kruskal-Wallis test. Correlations between clinical outcomes and biomarker levels were assessed via Spearman's correlation coefficient, with interpretations based on Cohen's guidelines. Statistical significance was set at $p \leq 0.05$.

Ethical considerations

The study was approved by the ethics committee of the medical faculty of RWTH Aachen University (EK 164/13) and conducted in accordance with the declaration of Helsinki as revised in 2013. Every participant gave written consent to participation of this study as well as interventions (surgery, lumbar puncture) after being briefed by a qualified physician. Surgical procedures were indicated following guidelines[4] regardless of participation in this study.

Results

Demographics and clinical disease burden

No significant differences in age, BMI, or gender distribution were observed among the DCMpre, DCMpost, and TAAA groups (Table 1). The mJOA scores were significantly different across groups, with higher scores in TAAA and an increase in DCMpost patients, suggesting partial recovery post-surgery. Disability indices (NDI and ODI) were markedly higher in DCM patients compared to controls, with no significant difference between DCMpre and DCMpost.

Variables	Groups			Significance
	DCM pre N=50	DCM post N=20	TAAA N=52	
	M [SD]	M [SD]	M [SD]	
Age	63 [11.2]	62.6 [11.4]	62.9 [14.1]	ns
Body-Mass-Index (BMI)	26.6 [5.0]	26.0 [4.4]	26.3 [5.1]	ns
mJOA Score	11 [3.1]	14 [2.0]	17 [1.3]	p<0.001
Neck disability Index (NDI)	40.2 [21.1]	37.4 [24.9]	6.1 [8.3]	p<0.001 for TAA vs. DCM pre and TAA vs. DCM post
Oswestry Disability Index (ODI)	41.4 [22.1]	38.8 [27.1]	7.3 [11.1]	p<0.001 for TAA vs. DCM pre and TAA vs. DCM post
Gender distribution				
female/male ratio	21/29	7/13	17/35	
Symptom onset	N [%]			
Duration <6 months		7 [35]		
Duration >6 months		13 [65]		

Tab. 1 Demographics.

CSF Angiopoetin 2 levels

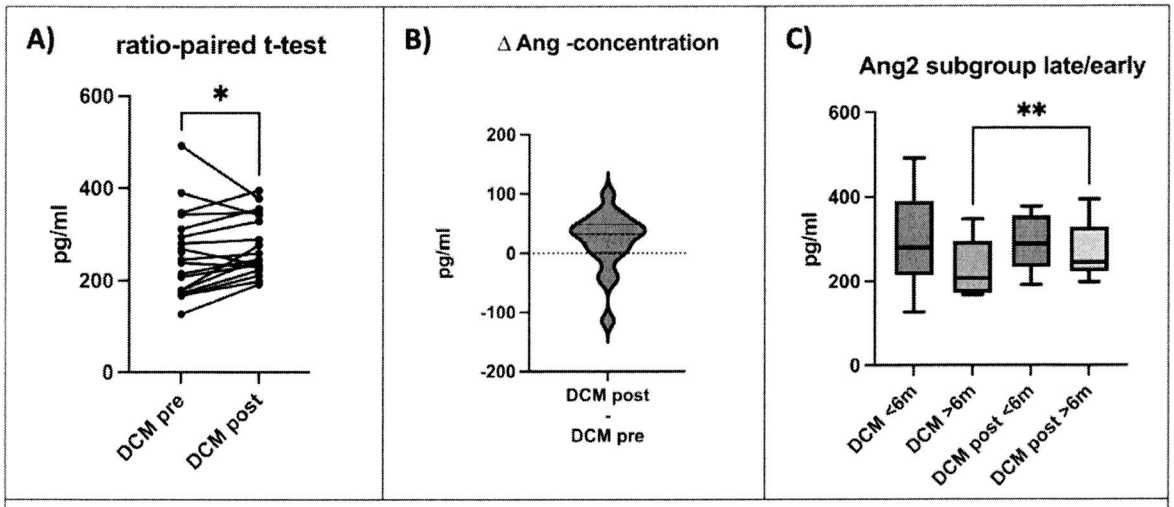

Fig. 2 ratio-paired t-test results

Ang2 concentrations were analyzed across groups, with some samples excluded due to pipetting errors. The mean Ang2 levels were 276 ± 90 pg/mL (DCMpre), 277 ± 65 pg/mL (DCMpost), and 463 ± 240 pg/mL in the TAAA group. ANOVA followed by Tukey's test showed significantly lower Ang2 levels in both DCM groups compared to TAAA ($p < 0.001$)(Fig.1). Although no significant difference was observed between DCMpre and DCMpost in overall mean Ang2 levels, a paired t-test in a subset of 16 patients showed a significant increase post-surgery ($p < 0.05$), especially in patients symptomatic for >6 (Fig.2).

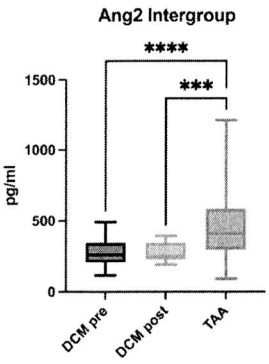

Fig. 1 One-way ANOVA results.

CSF VEGF-C levels

The technical nature of the Luminex assay does not require pipetting multiplicates. Although the test was pipetted in biological triplicates, pipetting errors did not necessarily lead to exclusion of data. VEGF-C levels were measured in 49 DCMpre, 19 DCMpost, and 48 TAAA patients. Mean VEGF-C levels were 189 ± 107 pg/mL (DCMpre), 234 ± 114 pg/mL (DCMpost), and 211 ± 114 pg/mL (TAAA). Kruskal-Wallis analysis indicated no significant differences between groups. Additional analyses, including ANOVA and subgroup analysis based on symptom duration, also revealed no significant group differences.

Correlation between CSF levels and clinical burden

Preoperative Ang2 levels showed a moderate negative correlation with NDI ($r = -0.46$; $p < 0.001$) and ODI ($r = -0.44$; $p < 0.001$), suggesting that lower Ang2 levels are associated with greater disability. However, no significant correlation was found between Ang2 levels and mJOA score changes post-surgery. VEGF-C levels did not correlate with clinical measures.

A detailed overview regarding the changes in Ang2 levels and mJOA scores of the 16 patients that yielded pre and postoperative results can be found in Tab. 2.

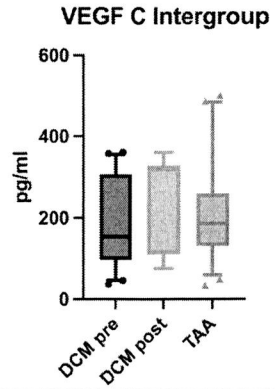

Fig. 3 Kruskal-Wallis results VEGF-C.

	DCM_{pre}		DCM_{post}		Recovery 3 months post surgery	
Patient No	**mJOA**	**$Ang2_{pg/ml}$**	**mJOA**	**$Ang2_{pg/ml}$**	**ΔmJOA**	**Δ $Ang2_{pg/ml}$**
1	12	178,52	12	277,09	0	98,57
2	7	189,07	13	246,08	4	57,01
3	17	167,06	17	211,258	0	44,2
4	15	171,72	15	198,14	*0*	26,42
5	16	245,7	16	259,2	0	13,5
6	12	171,67	15	223,29	3	51,62
7	16	208,15	17	235,1	1	26,95
8	11	214,6	12	246,48	1	31,88
9	10	294,56	13	328,7	3	34.14
10	15	126,5	16	191,73	1	65,23
11	11	311,77	15	355,73	4	43,96
12	11	347,43	13	394,61	2	47,18
13	9	280,15	11	288,96	2	8,81
14	11	269,07	16	234,46	5	-34,61
15	15	238,82	15	230,24	0	-8,58
16	12	343,27	11	347,89	-1	4,62

Discussion

Our findings indicate significantly lower CSF Ang2 levels in DCM patients compared to a control group of TAAA patients, with an increase in a subset of patients post-surgery. This aligns with the role of Ang2 in inflammation and vascular remodeling, highlighting its potential in modulating the inflammatory environment following spinal decompression. Preoperative Ang2 levels were correlated with neck and back pain-associated disability (NDI and ODI), but not with myelopathy severity (mJOA score). These results suggest that Ang2 may be a marker of pain-related disability rather than direct myelopathy severity.

Angiopetin-2 is a critical player in (neuro-)inflammation and angiogenesis primarily associated with vascular destabilization[6]. After traumatic (t)SCI, Ang2 expression is typically upregulated, which contributes to the disruption of endothelial cell junctions and increases vascular permeability[9,10]. This disruption facilitates the migration of immune cells into the spinal cord, which can exacerbate inflammation and secondary injury processes. However, Ang2 also plays a role in subsequent vascular remodeling and angiogenesis, which are crucial for repair and regeneration following SCI[9]. Our DCM cohorts featured decreased CSF Ang2 levels, indicating, that neuroinflammation in degenerative cervical myelopathy differ distinctively compared with traumatic SCI at time of indication for surgical decompression. Of course, there is a substantial difference in time between disease-/symptom-onset to diagnosis comparing DCM with tSCI. Although statistical significance was not reached, we observed a tendency towards lower Ang2 CSF levels in DCM patients that reported symptom-onset >6 months then those who reported that first symptoms appeared less then 6 months from surgical decompression (see Fig.2 C). Larger sample sizes might reveal a stronger time-dependent change of neuroinflammation in DCM.

Increased vascular permeability, or blood-spinal-cord-barrier (BSCB) disruption, is characteristic of DCM.[5,11,12]. In contrast to traumatic SCI models, our cohort of DCM patients featured decreased CSF concentrations of Ang2 compared with our control group of TAAA patients. This control group did not show a relevant BSCB disruption as measured by Reiber's diagnostics[12]. Therefore, Ang2 may not be the key factor maintaining BSCB disruption, at least not in DCM. Remarkably, we found Ang2 CSF levels

to increase following decompressive surgery possibly indicating, that relief from chronic spinal cord compression might normalize the inflammatory milieu in the intrathecal compartment. In a previous study of ours we were able to demonstrate a recovery of the BSCB, following decompressive surgery[11]. Furthermore, the magnitude of BSCB disruption correlates with symptom serverety[12]. We did not find a correlation between Ang2 levels and changes in ANG2 levels with symptom severity or recovery. Hence, Ang2 might rather function as an indirect inducer of change in vascular permeability and recovery, following mechanical compression and decompression, but downregulated during chronic state of compression, hypoperfusion and hypoxia of the spinal cord in DCM.

Conclusion

This study demonstrates lower CSF Ang2 levels in DCM patients relative to neurologically healthy controls, with a post-surgical increase, particularly in patients with symptom duration exceeding six months. Currently, Ang2 and VEGF-C levels are not viable biomarkers for symptom severity or recovery in DCM, though larger studies may reveal further insights into their role in disease onset and progression.

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Mittelverwendungsnachweis

Forschungsvorhaben: „Die sekundären Schädigungsmechanismen der degenerativen zervikalen Myelopathie“

Kennzeichnung: 36164

Projektleiter: Priv. Doz. Dr. med. Christian Blume

Bewilligungsbescheid:

Fördersumme: 1. Charge 7500 Euro 2. Charge noch nicht ausbezahlt

Projektdauer:

Einnahmen

Überweisung der Deutschen Wirbelsäulen Stiftung: 7500 Euro

Kassenbestand	280,72 Euro
Einnahmen gesamt	7.500,00 Euro

Ausgaben

Verbrauchsmittel:	
Neuroinflammation Panel Thermo Fischer 96 Tests (3x)	2.066,82 Euro
Neurodegeneration Panel Thermo Fischer 96 Tests (3x)	2.857,68 Euro
Angiopoetin-2 ELISA Kit 96 Tests (x3)	1.167,48 Euro
VEGF-C ELISA 96 Tests (x3)	1.127,30 Euro
Verbauchsmittel gesamt:	7.219,28 Euro

Ausgaben gesamt	7.219,28 Euro
Einnahmen gesamt	7.500,00 Euro
Kassenstand zum 31.12.2024	280,72 Euro

Der nicht verwendete Restmittelbestand wird an die Deutsche Wirbelsäulen Stiftung unter Angabe des Projektleiters und der Projekttitels zurückerstattet.

Bankverbindung: Südwestbank AG

IBAN DE 29 6009 0700 0414 7250 93

Abschließende Bemerkung

Wir möchten uns an dieser Stelle erneut für die Unterstützung der Deutschen Wirbelsäulen Stiftung bedanken. Es ist gelungen wissenschaftliche Ergebnisse herauszuarbeiten, die in der Wirbelsäulenforschung sicherlich ein Alleinstellungsmerkmal schaffen werden. Gerade in Hinblick auf die Erforschung von Grundlagenerkenntnissen bei komplexen Pathomechanismen wie der degenerativen zervikalen Myelopathie.

Vielen Dank und freundliche Grüße

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