Aus dem NeuroCure Clinical Research Center der
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DISSERTATION

Spinal cord atrophy measured from cerebral T1-weighted MRI: applications in clinical investigations of neuromyelitis optica spectrum disorders

zur Erlangung des akademischen Grades
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von

Claudia Chien
aus Hong Kong

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1. Synopsis

1.1 Abstract (English)

Magnetic resonance imaging (MRI) is used extensively for differential diagnosis and disease monitoring in neuroinflammatory disorders. Neuromyelitis optica spectrum disorders (NMOSD) are autoimmune neuroinflammatory diseases of the central nervous system that affect mainly the optic nerves, and spinal cord (SC). SC MRI shows longitudinally extensive transverse myelitis in the vast majority of NMOSD patients, where chronic inflammation leads to SC lesions and SC atrophy. In vivo imaging biomarkers are lacking for these patients, which could greatly aid in evaluating treatment efficacy or monitoring disease-related changes.

The key questions addressed in my dissertation are:

1. Can cerebral T1-weighted MRI be used to measure SC atrophy in NMOSD?
2. Is there a difference in SC affection between aquaporin-4 IgG seropositive (AQP4-IgG+) and myelin oligodendrocyte glycoprotein IgG seropositive (MOG-IgG+) NMOSD patients?
3. Is spinal cord atrophy associated with damage in thalamic subregions of AQP4-IgG+ patients with myelitis?

Cervical cord volume, total cord volume, and mean upper cervical cord area (MUCCA) were compared and demonstrated the ability to discriminate between AQP4-IgG+ NMOSD patients and healthy participants. MUCCA, measured from cerebral T1-weighted MRIs, correlated well with cervical cord and total cord volumes, even in patients. SC atrophy measurements using MUCCA were thus shown to accurately reflect damage in the entire SC of AQP4-IgG+ NMOSD patients. The SC lesion prevalence in specific locations and the mean MUCCA between the NMOSD antibody subgroups were similar. However, AQP4-IgG+ patients had more myelitis attacks, SC lesions, and SC atrophy than MOG-IgG+ patients. MUCCA associated with clinical attacks and disability in both NMOSD subgroups combined. Damage to the ventral posterior nucleus of the thalamus in AQP4-IgG+ patients with myelitis attacks were tested for association with MUCCA. This assessment of mutual damage/atrophy was negative. In summary, these studies proved that SC atrophy can be assessed using MUCCA, increases in NMOSD patients with more myelitis attacks, and that MUCCA is a valuable in vivo imaging parameter in NMOSD. These findings will be crucial in future clinical studies that monitor or evaluate treatment efficacy of patients with neuromyelitis optica spectrum disorders.

1.2 Abstract (Deutsch)

Die Magnetresonanztomographie (MRT) ist eine wichtige Methode für die Differentialdiagnose und das Monitoring neuroinflammatorischer Erkrankungen. Bei Neuromyelitis optica Spektrum-Erkrankungen (NMOSD) handelt es sich um eine Gruppe autoimmuner, inflammatorischer Erkrankungen des zentralen Nervensystems, die vor allem die Sehnerven und das Rückenmark betreffen. Mittels spinaler MRT kann bei einer überwiegenden Mehrheit der NMOSD Patienten eine longitudinale extensive transverse Myelitis nachgewiesen werden. Zur Beobachtung krankheitsbedingter Veränderungen in diesen Patienten und zur Therapieevaluation fehlen in-vivo bildgebende Biomarker.
Die vorliegende Dissertation behandelt drei zentrale Fragen anhand von Studien:

1. Eignen sich zerebrale T1-gewichtete MRT Aufnahmen, um die Rückenmarkatrophie bei NMOSD Patienten zu quantifizieren?
2. Gibt es Unterschiede zwischen Aquaporin-IgG-seropositiven (AQP4-IgG+) und Myelin-Oligodendrozyten-Glykoprotein (MOG-IgG+) NMOSD Patienten im Hinblick auf die Myelonaffektion?
3. Besteht bei AQP4-IgG+ Patienten mit Myelitis ein Zusammenhang zwischen Atrophie im Rückenmark und Schäden in Teilregionen des Thalamus?

Sowohl das Zervikalmarksvolumen, das gesamte Rückenmarksvolumen als auch die mittlere Querschnittsfläche des oberen Zervikalmarks (“mean upper cervical cord area”, MUCCA) zeigten signifikante Unterschiede zwischen AQP4-IgG+ NMOSD Patienten und gesunden Probanden. MUCCA, gemessen mittels zerebralen T1-gewichteten MRTs, korreliert gut mit den zuvor genannten Volumina, auch in NMOSD-Patienten. Die Quantifizierung von Rückenmarkatrophie mittels MUCCA ist daher geeignet, um Schäden im gesamten Rückenmark in AQP4-IgG+ NMOSD Patienten erfassen. Die Prävalenz von SC-Läsionen an bestimmten Lokationen und den MUCCA-Mittelwerten zwischen den NMOSD-Antikörper-Patientengruppen waren ähnlich. Im Vergleich zu MOG-IgG+ Patienten erleiden AQP4-IgG+ Patienten häufig mehr Myelitisschübe, Rückenmarksläsionen, und Rückenmarksatrophie. MUCCA korreliert mit der Anzahl der Krankheitsschübe und Behinderung für beide NMOSD Patientengruppen zusammen betrachtet. Eine Betrachtung der Schäden im Nucleus ventralis posterior des Thalamus in AQP4-IgG+ Patienten mit Myelitisschüben ergab keine Korrelationen mit MUCCA. Zusammenfassend konnte daher gezeigt werden, dass Rückenmarksatrophie mittels MUCCA gemessen werden kann, in NMOSD Patienten mit Myelitis die Atrophie zunimmt, und dass MUCCA einen wertvollen

in-vivo bildgebenden Biomarker darstellt. Diese Ergebnisse werden in der Durchführung zukünftiger klinischer Studien zur Beobachtung und Evaluierung von Therapien bei Patienten mit NMOSD eine wichtige Rolle spielen.

1.3 Introduction

Neuromyelitis optica spectrum disorders (NMOSD) are a group of rare autoimmune disorders of the central nervous system (CNS), where patients commonly present with optic neuritis, myelitis, and more rarely, brainstem and cerebral attacks [1]. Originally thought to be a subset of multiple sclerosis (MS), and often called Devic's Disease, its designation as a disease distinct from MS can be attributed to the finding that a pathogenic immunoglobulin-G specific for aquaporin-4 water channel protein is present in the serum of ~70% of patients (AQP4-IgG+) [2,3]. AQP4-IgG acts on astrocytic foot processes in CNS regions rich in AQP4 protein, such that demyelination is not the first inflammatory consequence in these NMOSD patients [4]. Some AQP4-IgG seronegative NMOSD patients are seropositive for antibodies against myelin-oligodendrocyte-glycoprotein (MOG-IgG+) [5]. MOG-IgG acts on oligodendrocytes, leading to both oligodendrocyte and myelin damage in the CNS of these patients, thus, complicating research into NMOSD patients further [6].

Although there are different immunological mechanisms in NMOSD patients that are AQP4-IgG+ versus MOG-IgG+, spinal cord (SC) affection, in the form of longitudinally extensive transverse myelitis (LETM), is common in both NMOSD phenotypes [7–9]. LETM can be seen as T2-hyperintense lesions in SC MRIs, spanning greater than 3 vertebral segments in about 85 – 90% of AQP4-IgG+ patients [10]. Chronic LETM or acute and/or shorter lesions can subsequently cause SC atrophy [11]. SC affection is a major component in patient prognosis, where length of myelitis-lesions have been shown to be associated with long-term disability in AQP4-IgG+ patients [12]. NMOSD myelitis attacks are often severe with incomplete recovery [10], thus it would be beneficial to these patients if there was an easy, reliable, *in vivo* method for measuring and monitoring SC atrophy. As a consequence, SC atrophy is increasingly being investigated in neuroinflammatory diseases by way of magnetic resonance imaging (MRI) [13].

One of the most common methods of SC atrophy measurement is the mean upper cervical cord area (MUCCA) [14]. Although MUCCA has been used in MS studies, there is no consensus on a particular method in calculating this measure and which MRI sequence to use. Selection of MRI sequence, as well as methods for analysis of SC atrophy need to be considered carefully during the planning stage of a prospective study, especially those involving limited MRI sessions or patients (e.g. in the case of rare diseases). MUCCA calculated from different MRI sequences or using different regions of the cord would likely lead to incomparable results [15]. Therefore, these factors cannot be overlooked during the

planning of multi-centered studies or clinical measurement of MUCCA, as this could lead to incorrect conclusions.

Since there are different immunological aspects to this disease, there are currently no established imaging biomarkers in NMOSD for monitoring disease stage or severity, or which measures could serve as outcomes in clinical trials [16]. SC MRI could lead to a clinically relevant imaging outcome measure in NMOSD, since lesions/LETM (location and length) can be seen using T2-weighted SC specific MRI [10]. Meanwhile, to measure atrophy of the SC, MUCCA calculated from T1-weighted cerebral MRIs [17,18] can be used, however, further evaluation of conventional SC MRI methods are required in NMOSD. This dissertation details the studies conducted to answer several important questions in relation to SC atrophy measurement and its application in clinical investigations of NMOSD patients.

1.3.1 Questions and Hypotheses

Three studies were conducted to answer the following research questions.

1. Can cerebral T1-weighted MRI be used to measure SC atrophy in NMOSD?
   In Study 1 [19] of this dissertation, it was hypothesized that MUCCA, measured from T1-weighted cerebral MRI, is robust and reflects the SC atrophy or damage in the entire spinal cord, therefore simplifying SC atrophy evaluation with respect to acquisition and measurement. We also tested whether different SC MRI measures could discriminate between healthy participants and NMOSD patients to further evaluate the clinical feasibility of using SC measurements to investigate disease-related structural damage in this set of diseases.

2. Is there a difference in SC affection between aquaporin-4 IgG seropositive (AQP4-IgG+) and myelin oligodendrocyte glycoprotein IgG seropositive (MOG-IgG+) NMOSD patients?
   Since the target of AQP4-IgG are mainly at the foot-processes of astrocytes, it would be expected that structural damage would be more severe in this disease subgroup. While MOG-IgG targets oligodendrocytes, it would be expected that myelin damage is the primary source of affection in these patients, which may not immediately present as atrophy in the CNS. Thus, the hypothesis in Study 2 [20] of this dissertation was that there would be a difference between AQP4-IgG+ and MOG-IgG+ NMOSD patient SC lesion prevalence and location, as well as SC atrophy.

3. Is there simultaneous atrophy in the ventral posterior nuclei of the thalamus and SC in AQP4-IgG+ NMOSD patients with myelitis?

   The thalamus has been found to be affected in neuroinflammatory diseases, such as multiple sclerosis, and since it receives and sends signals to many regions of the CNS, it has been proposed as an imaging marker in NMOSD as well. Thus, to evaluate whether NMOSD-related myelitis attacks are causing damage locally, or are able to spread to different regions of the CNS that are highly connected functionally and structurally to the spinal cord, MUCCA and thalamic volume were measured and compared. It was hypothesized in Study 3 [21] of this dissertation that the ventral posterior nucleus (VPN) of the thalamus would be atrophied at the same time and associate with SC atrophy due to myelitis.

1.4 State of Research

1.4.1 Spinal Cord Atrophy – MRI Context

Most studies on SC atrophy in NMOSD have focused on the measurement of cervical cross-sectional area. This is likely due to two reasons: 1) NMOSD lesions are most prevalent in the cervical and upper thoracic spinal cord [13] and 2) these measurements can be performed relatively simply. For example, it is possible to measure this parameter using a short MRI axial spine sequence. Furthermore, a 3D magnetization prepared rapid gradient echo (MPRAGE) MRI sequence of the brain, including the upper cervical cord (UCC), is able to give good agreement between MUCCA values in multi-centered studies [18]. Full or dedicated SC MRI scanning requires a significant amount of additional time and costs for patient visits. Complex analysis methods for the quantification of SC measures further decrease the availability of full SC evaluation in clinical routine. New sequences are available for SC myelin content or functional assessment in patients, but due to the experimental nature of the scanning and analysis, it has been difficult to implement into normal clinical routine [22].

However, the additional benefit of full SC evaluation, using commonly acquired 2D SC MRI, over a simple MUCCA measurement from cerebral MRIs was not previously investigated. The use of cerebral 3D MRIs with the UCC as a source for MUCCA calculations was chosen for Study 1 of this dissertation because many centers use this sequence to regularly image patients for detection of abnormalities in the brain. Both cerebral MPRAGE and T2-weighted full SC MRIs had been collected cross-sectionally in a relatively large cohort of AQP4-IgG+ NMOSD and healthy controls (HC) already, thus allowing for retrospective analysis of SC

atrophy. Therefore, full cervical cord and full cord volumes could be compared to MUCCA in an age- and sex-matched cohort, allowing for evaluation of MUCCA in detecting SC atrophy and discriminating between HC and NMOSD patients [19].

1.4.2 Spinal Cord Atrophy – Clinical Context

Myelitis, presenting in the form of LETM, has been reported in both patients with AQP4-IgG+ and MOG-IgG+ autoimmunity and is a common clinical NMOSD phenotype [23]. Acute and radiologically visible extensive SC inflammation and concurrent clinical myelitis attacks can subsequently cause SC atrophy [11]. SC atrophy measurements, especially MUCCA, are reduced in AQP4-IgG+ NMOSD compared to HC and lower MUCCA have been shown to be associated with worse clinical disability [13,24]. Quantification of SC damage is also becoming an important method to further investigate pathomechanisms in the different NMOSD subtypes [25]. However, large cohorts of NMOSD patients with definite AQP4-IgG and MOG-IgG seropositivity, identified with cell-based assays, were not previously compared in the context of SC affection by myelitis/LETM, lesions or atrophy. In Study 2 of this dissertation, we used SC specific MRI sequences to evaluate SC lesion lengths and locations, as well as cerebral MPRAGE MRI to measure MUCCA in AQP4-IgG+ and MOG-IgG+ NMOSD patients against HC [20].

1.4.3 Spinal Cord Atrophy – Non-focal Damage

Assessment of distal CNS regions from localized attacks has not been performed in this complex disease. The thalamus has been shown to be a major hub for inputs from sensory pathways (e.g. from the spinal cord) and subcortical regions of the brain, as well as from the cortex [26]. Atrophy of the total thalamus has been investigated with varied findings in NMOSD, where some groups have found total thalamic atrophy with clinical disability associations while others have not [27,28]. This could be due to differences in cohort selection, methods, or true differences in patient cohorts due to demographics. However, an in-depth investigation of the thalamic subregions has not been thoroughly performed in AQP4-IgG+ NMOSD patients. Especially in AQP4-IgG+ NMOSD, where the optic nerves and spinal cord (SC) are the primary sites of damage when patients present with an optic neuritis or myelitis attack [1], could evaluation and monitoring of these small regions be clinically relevant.

In this way, an association of the ventral posterior nuclei (VPN) of the thalamus with MUCCA in AQP4-IgG+ patients would serve as evidence of anterograde degeneration from

local attack regions to the thalamus. In Study 3 of this dissertation, we assessed the association of MUCCA, as a measure of SC atrophy, with VPN volume in patients compared to HC [21].

1.4.4 Objectives

This dissertation aimed to translate SC MRI methods to NMOSD research and to bridge the technical and clinically relevant applications of these methods. This involved the validation of using a common cerebral MRI sequence for SC atrophy measurements, representative of full SC damage. The validated method was then applied to studies which evaluated and elucidated new information on SC atrophy and affection in NMOSD patients. The overarching goal of this research is to have an MRI biomarker, which can easily be implemented using common clinical MRI scans and is indicative of and/or can be used to monitor NMOSD patient disability severity and extent of tissue damage.

1.5 Materials and Methods

This section gives an overview of the main MRI techniques utilized in this dissertation. Specific study methodologies, including study design, patient demographics and selection criteria, image analysis, and statistical analysis are described in full in the attached original publications [19–21].

All data were derived from two retrospective, observational, ongoing studies: the Neuromyelitis Optica (NMO) study and the Visual Imaging Multiple Sclerosis (VIMS) study at the NeuroCure Clinical Research Center (NCRC), Charité-Universitätsmedizin Berlin (EA1/041/14 and EA1/163/12). All studies were approved by the ethics committee of the Charité-Universitätsmedizin Berlin. All MRI scanning was performed on a 3T Siemens Tim Trio machine at the Berlin Center for Advanced Neuroimaging (BCAN), across from the NCRC in the Charité-Campus Mitte. Patients gave written informed consent to participate in the studies. After validation and peer-reviewed publications using the MUCCA analysis technique, MUCCA is now incorporated into all observational studies within the routine post-processing pipelines.

1.5.1 Evaluation of MUCCA as a surrogate measure for cervical and full spinal cord atrophy in AQP4-IgG+ NMOSD patients

Spinal cord (SC) changes and involvement have been shown to be an important factor in patient disability prognosis in neuromyelitis optica spectrum disorders (NMOSD) and

multiple sclerosis (MS) [29,30]. Due to the nature of MRI, there are limitations in the sensitivity and specificity when imaging and analyzing the SC, especially in disease-states that are not clearly understood, such as in NMOSD. This objective was addressed in the first publication (Study 1) within this dissertation [19]. In MS, patients often have decreased SC cross-sectional areas as measured by MRI, that indicates axonal degeneration, which is a secondary effect in the disease after demyelination [31]. However, in aquaporin-4 immunoglobulin-G seropositive (AQP4-IgG+) NMOSD patients, it is thought that disease-related attacks and lesions are caused by a primary astrocytopathy [32], thus it is unknown whether short-term/cross-sectional analysis of SC cross-sectional areas are able to detect changes in these patients.

To show that measuring the mean upper cervical cord area (MUCCA) from cerebral MRI scans is a reliable and representative measure of SC atrophy in NMOSD, we conducted Study 1 with 30 AQP4-IgG+ NMOSD patients and 19 healthy controls. We used 2D T2-weighted SC MRI sequences to calculate cervical cord and full SC volumes, while 3D T1-weighted magnetization prepared rapid acquisition of gradient echo (MPRAGE) brain scans including the upper cervical cord were used to calculate MUCCA.

Total cord volume (TCV) was measured from SC MRIs at 3 different levels: cervical, thoracic, and lumbar. The volumetric analysis of the full SC required the addition of 3 segments measured from: 1) the tip of the dens to the rostral border of the thoracic (T)1 vertebral body, 2) the rostral border of the T1 vertebral body to the T11 vertebral body rostral border, and 3) the T11 vertebral body rostral border to the conus tip. Figure 1 [19], shows sample segmentations of MUCCA, cervical cord volume (CCV), and representative segmentations of the thoracic and lumbar SC.

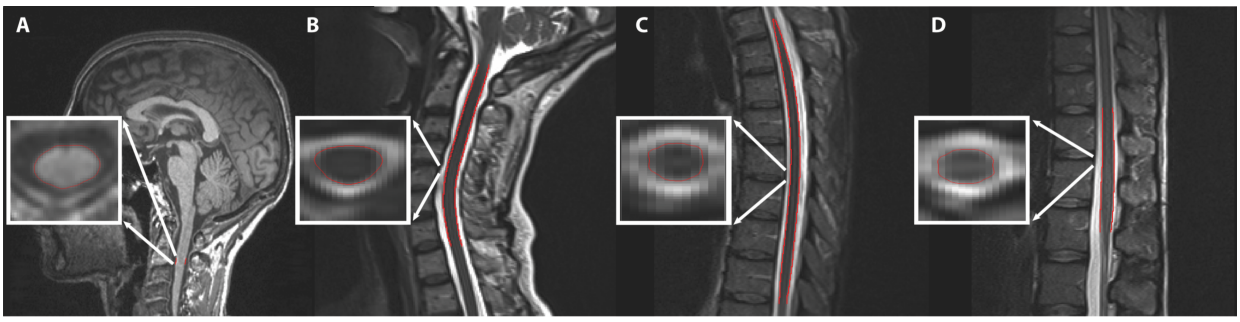

Figure 1. *Sample segmentation of A) MUCCA from a T1-weighted cerebral MPRAGE sequence, B) cervical cord volume, C) the thoracic SC volume, and D) the lumbar SC volume calculated from T2-weighted SC sequences. The total cord volume is calculated from the sum of B, C, and D. Adapted from Chien et al., AJNR, 2018 [19].*

Group differences in discrete measures were tested with a Chi-squared test, while for continuous measurements (i.e. age, MUCCA, CCV, TCV) a two-sampled t-test was used. MUCCA, CCV, and TCV correlations with each other and with parametric clinical measures, such as averaged timed 25-foot walk and dominant and nondominant hand 9-hole peg test times were tested using a Pearson's correlation test. Correlations with discrete measures were analyzed with a Spearman rank correlation test. Performance of each SC measure was evaluated using receiver operating characteristic analysis for the detection of NMOSD versus HC SC measures.

1.5.2 Evaluation of SC affection in AQP4-IgG+ versus MOG-IgG+ NMOSD patients

Autoantibodies against aquaporin-4 (AQP4) water channel proteins mainly target astrocytes, the choroid plexus epithelial cells and possibly Müller cells in the retina. Meanwhile, autoantibodies against myelin oligodendrocyte glycoprotein (MOG) predominantly attach to the outermost layers of myelin sheaths [33]. Recently, there have been many discussions over whether or not MOG-IgG+ patients should be categorized as having NMOSD at all [34,35]. Nevertheless, studies have found similar pathological features regarding SC involvement and clinical manifestations in both patients seropositive for AQP4-IgG and MOG-IgG [9]. However, validation of differences in SC affection and their clinical impact between the two antibody subgroups of patients with NMOSD have not previously been assessed. This was most likely caused by the relatively new diagnostic criteria for NMOSD, including lack of availability and costs for MOG-IgG cell-based assays [1,36]. This objective was addressed in the second publication (Study 2) within this dissertation. Several clinical measures may affect the SC atrophy observed in NMOSD: the number of clinical myelitis attacks and the time since last myelitis attack. Since it is hypothesized that severe acute clinical attacks in NMOSD are the cause of immediate axonal destruction within lesions [9,37], it is pertinent to explore this theme further by looking at atrophy measures in relation to the time since a last myelitis attack.

In Study 2 [20], we endeavoured to evaluate and validate differences between AQP4-IgG+ and MOG-IgG+ NMOSD (cell-based assay serostatus confirmed) patient SC abnormalities by recording T2-hyperintense SC lesion locations, length, and counts. We also calculated the mean upper cervical cord area (MUCCA), as a measure of SC atrophy, in each patient subgroup and compared these measures with age- and sex-matched healthy controls (HC). Clinical manifestation was tested for correlations with MUCCA, using disease duration, clinical myelitis attack counts, the expanded disability status scale (EDSS) score, the

pyramidal function score (a section of the Neurostatus EDSS score [38]), the timed 25-foot walk test times, and 9-hole peg test times. A total of 53 NMOSD (AQP4-IgG+ n = 38; MOG-IgG+ n = 15) patients were included in the study. The MRIs utilized in this study included a 2D T2-weighted SC sequence to visualize and calculate SC lesions locations, lengths, and counts, and a T1-weighted 3D MPRAGE brain MRI including the upper cervical cord for MUCCA measurement.

SC lesion counts and median lesion lengths were analyzed for differences using Chi-square tests, while MUCCA group differences between HC and NMOSD patients were calculated using Welch's two-sample t-test. Correlations between clinical myelitis attack numbers and weeks since last myelitis with MUCCA, were performed using Spearman's rho estimate. Clinical manifestations of SC atrophy were evaluated using age and head-size as covariates with parametric multivariable linear models.

1.5.3 Myelitis attack related changes in the ventral posterior thalamic nuclei in association with MUCCA of AQP4-IgG+ NMOSD patients

Although normal (non-atrophied) deep grey matter volumes have been seen in NMOSD patients compared to healthy controls [28], it has not been investigated previously whether subtle changes are occurring in subregions of the thalamus. This objective was addressed in the third publication (Study 3) within this dissertation [21]. Due to recent advances in MRI analysis methods, there are now advanced 3D atlases based on histological data, which fit each anatomical region of the basal ganglia, including subregions (nuclei) of the thalamus [39]. Since the thalamus is connected to cortical regions [26], and several nuclei of this basal ganglia component receive direct signals from the visual and spinothalamic pathways [40], it would be expected that any anterograde or retrograde degeneration along these tracks would be seen in these structures. Evaluation of damage from myelitis in the non-localized region of the ventral posterior nucleus (VPN) of the thalamus would allow for evaluation of this type of degeneration.

In Study 3, we performed a cross-sectional analysis of 39 patients with AQP4-IgG+ NMOSD and 37 age- and sex-matched healthy controls (HC). 3 Tesla T1-weighted 3D magnetization prepared rapid gradient echo (MPRAGE) brain including the upper cervical cord was used for mean upper cervical cord area (MUCCA) calculation. T2-hyperintense lesion masks were made with T2-weighted 3D fluid attenuated inversion recovery (FLAIR) brain MRIs, which allowed for lesion-infilling of brain lesion voxel intensities in each patient MPRAGE scan. This step is necessary to increase accuracy of the atlas-based segmentation of the deep grey

matter, in the event that lesions are in and around these structural brain components. The Multiple Automatically Generated Templates Brain Segmentation Algorithm (MAGeTbrain, http://cobralab.ca/software/MAGeTbrain/) was used for segmentation of the thalamus and its subregions in each individual participant MRI scan, giving volumes for the VPN. Associations of VPN volumes with demographic, clinical, and MUCCA measures were evaluated using linear mixed effect models (LMM), with inter-side and intra-participant dependencies, adjusting for age and sex as fixed effects.

1.6 Results

1.6.1 Evaluation of MUCCA as a surrogate measure for cervical and full spinal cord atrophy in AQP4-IgG+ NMOSD patients

In Study 1 [19], we demonstrated that all 3 types of SC quantification could be used to differentiate between age- and sex-matched HC (n = 19) and AQP4-IgG+ NMOSD patients (n = 30), where all measures were significantly dependent on each other. Receiver operating characteristic analysis was used for evaluating accuracy and sensitivity in each measure's ability to discriminate between HC and NMOSD, which showed similar areas under the curve (Figure 2). Table 1 illustrates how all 3 SC quantification methods show statistically different measures between HC and patients (adapted from Table 2 in Chien et al. AJNR, 2018 [19]) using two-sampled t-tests

Mean upper cervical cord area (MUCCA), the cervical cord volume, and total cord volume did not correlate with clinical disability, as measured by the expanded disability status scale, the pyramidal functional system score, and the averaged timed 25-foot walk and 9-hole peg tests.

Table 1 | Quantitative SC MRI measures

SC Measure	Healthy Controls (mean ± SD)	AQP4-IgG+ NMOSD patients (mean ± SD)	t-statistic; p-value
MUCCA (mm^2)	73.3 ± 5.51	68.5 ± 7.06	2.70; .009*
CCV (mL)	7.52 ± 0.92	6.61 ± 0.96	3.33; .002*
TCV (mL)	20.1 ± 2.37	17.6 ± 2.21	3.69; <.001*

NMOSD = neuromyelitis optica spectrum disorders; MUCCA = mean upper cervical cord area; CCV = cervical cord volume; TCV = total cord volume; SD = standard deviation; *=statistical difference from healthy subjects ($p < .01$). Adapted from Chien et al., AJNR, 2018.

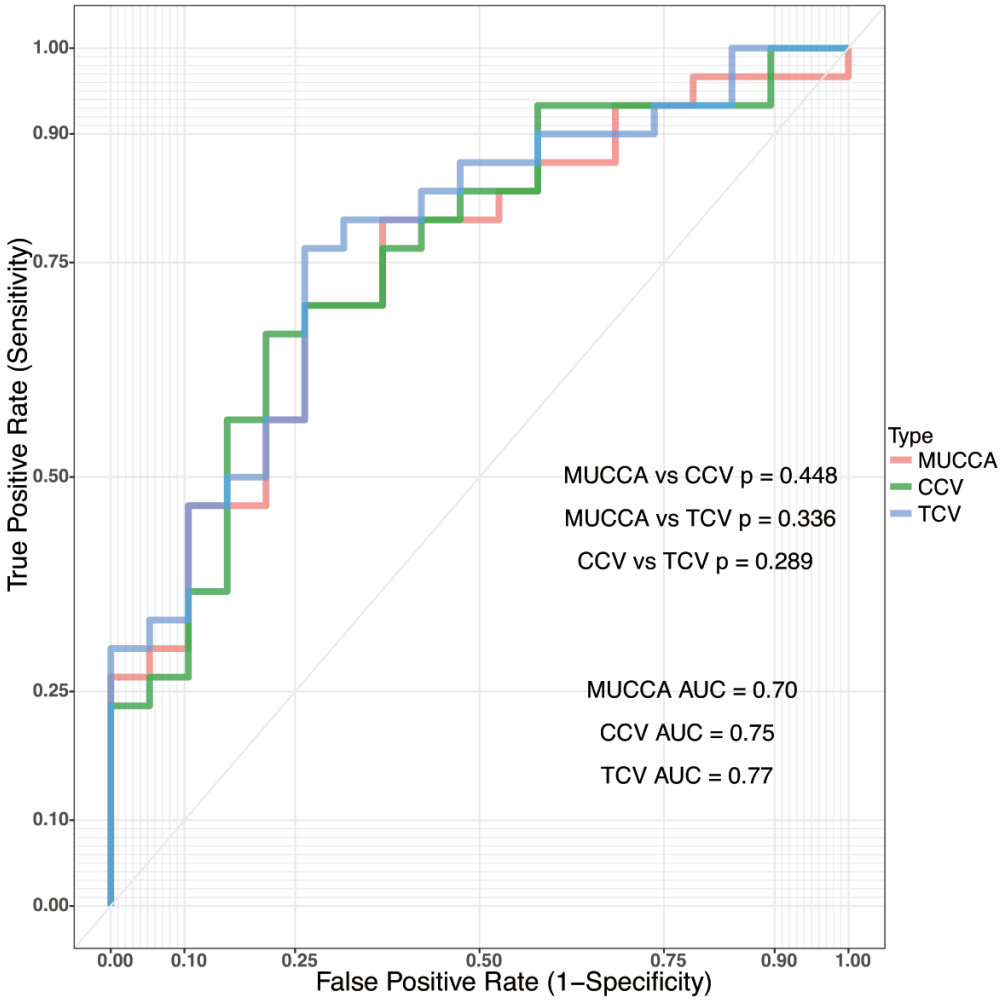

Figure 2. *Receiver operating characteristics showing the discriminatory power of the mean upper cervical cord area, cervical cord volume, and total cord volume for identification of AQP4-IgG+ NMOSD patients versus healthy controls. Abbreviations: MUCCA = mean upper cervical cord area, CCV = cervical cord volume, TCV = total cord volume, AUC = area under the curve. Adapted from Chien et al., AJNR, 2018 [19]*

1.6.2 Evaluation of SC affection in AQP4-IgG+ versus MOG-IgG+ NMOSD patients

In Study 2 [20], investigating SC affection in AQP4-IgG+ (n = 38) and MOG-IgG+ (n = 15) NMOSD patients, we found: 1) a higher history of myelitis and prevalence of SC lesions in AQP4-IgG+ NMOSD; and 2) that higher counts of clinical myelitis attacks were associated with decreased MUCCA in all NMOSD patients. We could not statistically evaluate the effect of treatment on MUCCA in these patients, due to the variety of attack preventing therapies given (Figure 3), however, it can be seen that most patients had a decreased MUCCA compared to HC, as long as they had a history of myelitis attacks, regardless of treatment.

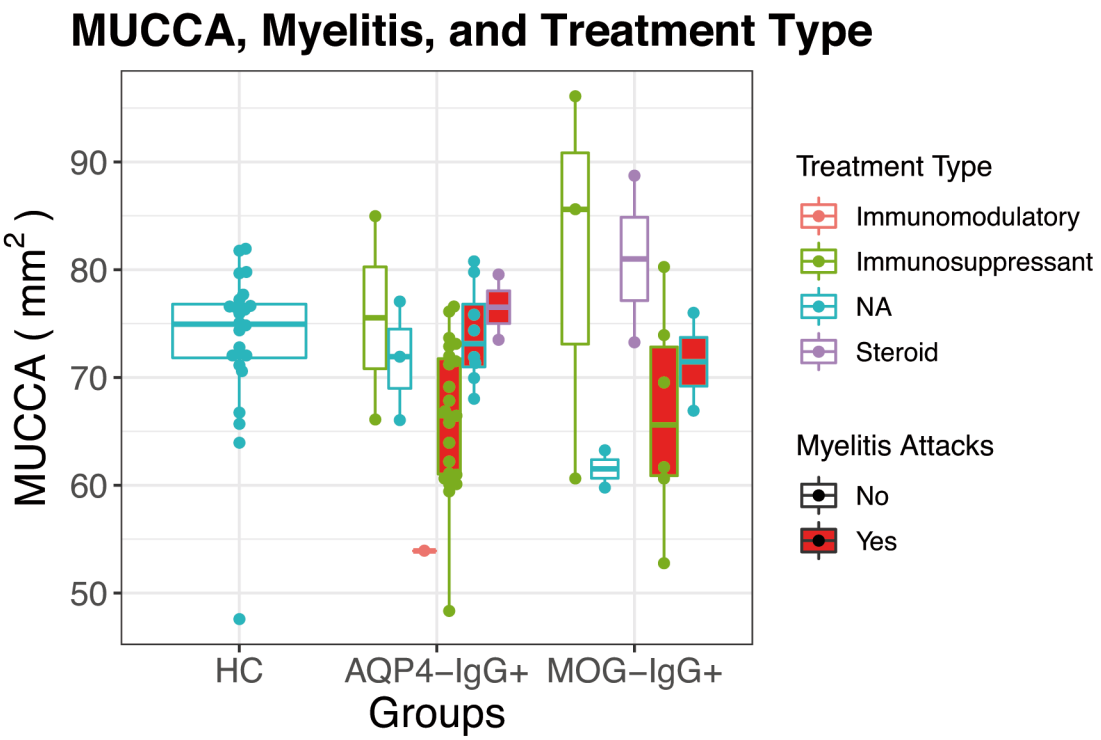

Figure 3. *MUCCA from HC, AQP4-IgG+, and MOG-IgG+ NMOSD patients with associated attack preventing therapies. Abbreviations: HC = healthy controls, AQP4-IgG+ = aquaporin-4 IgG seropositive NMOSD, MOG-IgG+ = myelin oligodendrocyte glycoprotein IgG seropositive NMOSD, MUCCA = mean upper cervical cord area, NA = not applicable/unknown. Chien et al., unpublished data.*

Finally, MUCCA had associations with clinical disability in both AQP4-IgG+ and MOG-IgG+ patients as measured by the expanded disability status scale, pyramidal functional systems score, averaged timed 25-foot walk and standardized 9-hole peg test ($p = 0.030$, $p = 0.003$, $p = 0.010$, and $p = 0.037$, respectively) when age and head-size were taken into account [20].

1.6.3 Myelitis attack related changes in the ventral posterior thalamic nuclei in association with MUCCA of AQP4-IgG+ NMOSD patients

In Study 3 [21], we evaluated whether or not the ventral posterior thalamic nuclei (VPN) volume in NMOSD patients decreased as a result of clinical myelitis attacks. The mean VPN volume was not different ($p = 0.730$) between age- and sex-matched AQP4-IgG+ NMOSD patients ($n = 39$) and HC ($n = 37$). No VPN volume differences between a subgroup of NMOSD patients with a history of myelitis and HC, nor any associations with the number of historical myelitis attacks were found. To evaluate associations of VPN volume with known

myelitis-related SC damage, MUCCA was used as a comparator. VPN volume was not associated with MUCCA in the entire patient cohort ($p = 0.261$) or in a subgroup of patients with a history of clinical myelitis attacks ($p = 0.084$). Finally, no correlation was found between VPN volume and the sensory functional systems score of the expanded disability status scale. However, VPN volume showed a trend to be decreased in patients with a history of clinical brainstem attacks versus HC [21].

1.7 Conclusions and Future Directions

From the studies conducted for this PhD dissertation, three overarching questions were answered:

1. Can cerebral T1-weighted MRI be used to measure SC atrophy in NMOSD?
   Yes, MUCCA measured from cerebral MPRAGE scans including the upper cervical cord is able to reliably evaluate SC atrophy, representative of total cord volume in NMOSD. Since MUCCA was able to discriminate between healthy participants and NMOSD patients [19], we deemed that this measure can be used to evaluate SC atrophy in our multiple sclerosis and NMOSD cohorts in further clinical studies from the Charité-Universitätsmedizin Berlin. However, further investigations into the longitudinal use of MUCCA must be assessed and tested in a larger cohort, prior to using this measure to investigate changes in the SC over time.
2. Is there a difference in SC affection between aquaporin-4 IgG seropositive (AQP4-IgG+) and myelin oligodendrocyte glycoprotein IgG seropositive (MOG-IgG+) NMOSD patients?
   Yes, there is a difference in SC affection between the two NMOSD subgroups, where AQP4-IgG+ NMOSD patients had more SC lesions and atrophy than MOG-IgG+ patients. We were able to show that decreased MUCCA is directly associated with an increased number of clinical myelitis attacks in both patient subgroups [20]. Thus, our findings suggest that there are differences in SC affection between the two antibody serostatus groups, which furthers our collective knowledge of attack-related damage and common MRI markers that can be used for monitoring patients with AQP4-IgG and MOG-IgG associated autoimmunity. Since SC affection is prevalent in both patient groups, we will continue to monitor lesions and atrophy in the cord using MRI, although it remains to be seen how this information may be used in the clinical context for patient treatment or disability assessment.

3. Is there simultaneous atrophy in the ventral posterior nuclei of the thalamus and SC in AQP4-IgG+ NMOSD patients with myelitis?
   No, there was no atrophy detected in the thalamic ventral posterior nuclei compared to healthy participants, although there was SC atrophy measured by MUCCA in NMOSD patients, which is in line with previous studies. This would suggest that CNS damage in AQP4-IgG+ NMOSD occurs due to clinical attacks, with higher severity in localized regions (i.e. myelitis in the SC causes atrophy in the SC). However, we could not completely discount any damage in the thalamic subregions due to clinical attacks, since volumetric measurements using 3 Tesla MRI may not have the sensitivity to identify microstructural changes, or other disease-related damage causing functional or adaptive changes [21].

Overall, the publications related to this dissertation revealed that MUCCA is a reliable and representative measure that is sensitive to SC atrophy in NMOSD. Using SC MRI, we were able to elucidate that NMOSD patients with increasing counts of clinical myelitis attacks accrue more SC atrophy; and that AQP4-IgG+ patients have a higher prevalence of myelitis attacks, SC lesions, and SC atrophy than MOG-IgG+ patients. We did not observe myelitis-related damage in the ventral posterior nucleus of the thalamus in conjunction with a decrease in MUCCA in AQP4-IgG+ NMOSD patients. In conclusion, MUCCA is a parameter which can detect NMOSD-related SC damage using 3 Tesla cerebral T1-weighted MRIs, and can be used to monitor and quantitatively measure atrophy of the SC in these patients.
Future directions would include the longitudinal monitoring of SC cross-sectional area in NMOSD to evaluate any disease progression or atrophy over time or treatment effects. It would also be interesting and beneficial to further investigate thalamic subregional volumes using higher magnet strengths (i.e. 7 Tesla MRI) to evaluate changes distal from attack regions, which are possibly associated with cognitive impairment.
In conclusion, the SC MRI quantitative measure investigated in this dissertation was not previously used in routine clinical observational studies, was shown to be feasible using a common MRI sequence, and is able to give valuable information about localized and overall damage in the SC of NMOSD patients. This technique will impact clinical trial planning when SC atrophy is a prospective outcome measure and may help us further investigate pathophysiological processes, and possibly evaluate patient prognoses and clinical outcomes in this rare spectrum of autoimmune diseases.

1.8 References

1. Wingerchuk DM, Banwell B, Bennett JL, Cabre P, Carroll W, Chitnis T, de Seze J, Fujihara K, Greenberg B, Jacob A, Jarius S, Lana-Peixoto M, Levy M, Simon JH, Tenembaum S, Traboulsee AL, Waters P, Wellik KE, Weinshenker BG. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology. 2015 Jul 14;85(2):177–89.

2. Devic E. Myélite subaigue compliquée de névrite optique. Bull Méd. 1894;8:1033–4.

3. Jarius S, Wildemann B. On the contribution of Thomas Clifford Allbutt, F.R.S., to the early history of neuromyelitis optica. J Neurol. 2013 Jan 1;260(1):100–4.

4. Jarius S, Paul F, Franciotta D, Waters P, Zipp F, Hohlfeld R, Vincent A, Wildemann B. Mechanisms of disease: aquaporin-4 antibodies in neuromyelitis optica. Nat Clin Pract Neurol. 2008 Apr;4(4):202–14.

5. Jarius S, Ruprecht K, Kleiter I, Borisow N, Asgari N, Pitarokoili K, Pache F, Stich O, Beume L-A, Hümmert MW, Ringelstein M, Trebst C, Winkelmann A, Schwarz A, Buttmann M, Zimmermann H, Kuchling J, Franciotta D, Capobianco M, Siebert E, Lukas C, Korporal-Kuhnke M, Haas J, Fechner K, Brandt AU, Schanda K, Aktas O, Paul F, Reindl M, Wildemann B, in cooperation with the Neuromyelitis Optica Study Group (NEMOS). MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 2: Epidemiology, clinical presentation, radiological and laboratory features, treatment responses, and long-term outcome. J Neuroinflammation. 2016 Sep 27;13(1):280.

6. Zamvil SS, Slavin AJ. Does MOG Ig-positive AQP4-seronegative opticospinal inflammatory disease justify a diagnosis of NMO spectrum disorder? Neurol Neuroimmunol Neuroinflammation. 2015 Feb;2(1):e62.

7. Cacciaguerra L, Meani A, Mesaros S, Radaelli M, Palace J, Dujmovic-Basuroski I, Pagani E, Martinelli V, Matthews L, Drulovic J, Leite MI, Comi G, Filippi M, Rocca MA. Brain and cord imaging features in neuromyelitis optica spectrum disorders. Ann Neurol. 2019;85(3):371–84.

8. Loos J, Pfeuffer S, Pape K, Ruck T, Luessi F, Spreer A, Zipp F, Meuth SG, Bittner S. MOG encephalomyelitis: distinct clinical, MRI and CSF features in patients with longitudinal extensive transverse myelitis as first clinical presentation. J Neurol [Internet]. 2020 Feb 13 [cited 2020 Mar 16]; Available from: https://doi.org/10.1007/s00415-020-09755-x

9. Ciccarelli O, Cohen JA, Reingold SC, Weinshenker BG, International Conference on Spinal Cord Involvement and Imaging in Multiple Sclerosis and Neuromyelitis Optica Spectrum Disorders. Spinal cord involvement in multiple sclerosis and neuromyelitis optica spectrum disorders. Lancet Neurol. 2019 Feb;18(2):185–97.

10. Zalewski NL, Morris PP, Weinshenker BG, Lucchinetti CF, Guo Y, Pittock SJ, Krecke KN, Kaufmann TJ, Wingerchuk DM, Kumar N, Flanagan EP. Ring-enhancing spinal cord lesions in neuromyelitis optica spectrum disorders. J Neurol Neurosurg Psychiatry. 2017 Mar;88(3):218–25.

11. Wang Y, Wang Y, Tan S, Lu Z. Spinal cord atrophy in neuromyelitis optica spectrum disorders. Mult Scler Relat Disord. 2016 Jul;8:9–10.

12. Mealy MA, Mossburg SE, Kim S-H, Messina S, Borisow N, Lopez-Gonzalez R, Ospina JP, Scheel M, Yeshokumar AK, Awad A, Leite MI, Arango JJ, Paul F, Palace J, Kim HJ, Levy M. Long-term disability in neuromyelitis optica spectrum disorder with a history of myelitis is associated with age at onset, delay in diagnosis/preventive treatment, MRI lesion length and presence of symptomatic brain lesions. Mult Scler Relat Disord. 2019 Feb;28:64–8.

13. Liu Y, Wang J, Daams M, Weiler F, Hahn HK, Duan Y, Huang J, Ren Z, Ye J, Dong H, Vrenken H, Wattjes MP, Shi F-D, Li K, Barkhof F. Differential patterns of spinal cord and brain atrophy in NMO and MS. Neurology. 2015 Apr 7;84(14):1465–72.

14. Alcaide-Leon P, Cybulsky K, Sankar S, Casserly C, Leung G, Hohol M, Selchen D, Montalban X, Bharatha A, Oh J. Quantitative spinal cord MRI in radiologically isolated syndrome. Neurol Neuroimmunol Neuroinflammation. 2018 Mar;5(2):e436.

15. Weeda MM, Middelkoop SM, Steenwijk MD, Daams M, Amiri H, Brouwer I, Killestein J, Uitdehaag BMJ, Dekker I, Lukas C, Bellenberg B, Barkhof F, Pouwels PJW, Vrenken H. Validation of mean upper cervical cord area (MUCCA) measurement techniques in multiple sclerosis (MS): High reproducibility and robustness to lesions, but large software and scanner effects. NeuroImage Clin. 2019 Aug 6;24:101962.

16. Weinshenker BG, Barron G, Behne JM, Bennett JL, Chin PS, Cree BAC, de Seze J, Flor A, Fujihara K, Greenberg B, Higashi S, Holt W, Khan O, Knappertz V, Levy M, Melia AT, Palace J, Smith TJ, Sormani MP, Van Herle K, VanMeter S, Villoslada P, Walton MK, Wasiewski W, Wingerchuk DM, Yeaman MR. Challenges and opportunities in designing clinical trials for neuromyelitis optica. Neurology. 2015 Apr 28;84(17):1805–15.

17. Losseff NA, Webb SL, O'Riordan JI, Page R, Wang L, Barker GJ, Tofts PS, McDonald WI, Miller DH, Thompson AJ. Spinal cord atrophy and disability in multiple sclerosis. A new reproducible and sensitive MRI method with potential to monitor disease progression. Brain J Neurol. 1996 Jun;119 (Pt 3):701–8.

18. Liu Y, Lukas C, Steenwijk MD, Daams M, Versteeg A, Duan Y, Li K, Weiler F, Hahn HK, Wattjes MP, Barkhof F, Vrenken H. Multicenter Validation of Mean Upper Cervical Cord Area Measurements from Head 3D T1-Weighted MR Imaging in Patients with Multiple Sclerosis. AJNR Am J Neuroradiol. 2016 Apr;37(4):749–54.

19. Chien C, Brandt AU, Schmidt F, Bellmann-Strobl J, Ruprecht K, Paul F, Scheel M. MRI-Based Methods for Spinal Cord Atrophy Evaluation: A Comparison of Cervical Cord Cross-Sectional Area, Cervical Cord Volume, and Full Spinal Cord Volume in Patients with Aquaporin-4 Antibody Seropositive Neuromyelitis Optica Spectrum Disorders. AJNR Am J Neuroradiol. 2018 Jul;39(7):1362–8.

20. Chien C, Scheel M, Schmitz-Hübsch T, Borisow N, Ruprecht K, Bellmann-Strobl J, Paul F, Brandt AU. Spinal cord lesions and atrophy in NMOSD with AQP4-IgG and MOG-IgG associated autoimmunity. Mult Scler Houndmills Basingstoke Engl. 2019 Dec;25(14):1926–36.

21. Papadopoulou A, Oertel FC, Gaetano L, Kuchling J, Zimmermann H, Chien C, Siebert N, Asseyer S, Bellmann-Strobl J, Ruprecht K, Chakravarty MM, Scheel M, Magon S, Wuerfel J, Paul F, Brandt AU. Attack-related damage of thalamic nuclei in neuromyelitis optica spectrum disorders. J Neurol Neurosurg Psychiatry. 2019 Oct 1;90(10):1156–64.

22. Martin AR, Aleksanderek I, Cohen-Adad J, Tarmohamed Z, Tetreault L, Smith N, Cadotte DW, Crawley A, Ginsberg H, Mikulis DJ, Fehlings MG. Translating state-of-the-art spinal cord MRI techniques to clinical use: A systematic review of clinical studies utilizing DTI, MT, MWF, MRS, and fMRI. NeuroImage Clin. 2016;10:192–238.

23. Sato DK, Callegaro D, Lana-Peixoto MA, Waters PJ, de Haidar Jorge FM, Takahashi T, Nakashima I, Apostolos-Pereira SL, Talim N, Simm RF, Lino AMM, Misu T, Leite MI, Aoki M, Fujihara K. Distinction between MOG antibody-positive and AQP4 antibody-positive NMO spectrum disorders. Neurology. 2014 Feb 11;82(6):474–81.

24. Schneider R, Bellenberg B, Kleiter I, Gold R, Köster O, Weiler F, Hahn H, Lukas C. Cervical cord and ventricle affection in neuromyelitis optica. Acta Neurol Scand. 2016 Apr 21;

25. Combes AJE, Matthews L, Lee JS, Li DKB, Carruthers R, Traboulsee AL, Barker GJ, Palace J, Kolind S. Cervical cord myelin water imaging shows degenerative changes over one year in multiple sclerosis but not neuromyelitis optica spectrum disorder. NeuroImage Clin. 2017;16:17–22.

26. Hwang K, Bertolero MA, Liu WB, D'Esposito M. The Human Thalamus Is an Integrative Hub for Functional Brain Networks. J Neurosci. 2017 Jun 7;37(23):5594–607.

27. Hyun J-W, Park G, Kwak K, Jo H-J, Joung A, Kim J-H, Lee SH, Kim S, Lee J-M, Kim S-H, Kim HJ. Deep gray matter atrophy in neuromyelitis optica spectrum disorder and multiple sclerosis. Eur J Neurol. 2017;24(2):437–45.

28. Finke C, Heine J, Pache F, Lacheta A, Borisow N, Kuchling J, Bellmann-Strobl J, Ruprecht K, Brandt AU, Paul F. Normal volumes and microstructural integrity of deep gray matter structures in AQP4+ NMOSD. Neurol Neuroimmunol Neuroinflammation. 2016 Jun;3(3):e229.

29. Kim HJ, Paul F, Lana-Peixoto MA, Tenembaum S, Asgari N, Palace J, Klawiter EC, Sato DK, de Seze J, Wuerfel J, Banwell BL, Villoslada P, Saiz A, Fujihara K, Kim S-H, Guthy-Jackson Charitable Foundation NMO International Clinical Consortium & Biorepository. MRI characteristics of neuromyelitis optica spectrum disorder: an international update. Neurology. 2015 Mar 17;84(11):1165–73.

30. Brownlee WJ, Altmann DR, Alves Da Mota P, Swanton JK, Miszkiel KA, Wheeler-Kingshott CG, Ciccarelli O, Miller DH. Association of asymptomatic spinal cord lesions and atrophy with disability 5 years after a clinically isolated syndrome. Mult Scler Houndmills Basingstoke Engl. 2017 Apr;23(5):665–74.

31. Petrova N, Carassiti D, Altmann DR, Baker D, Schmierer K. Axonal loss in the multiple sclerosis spinal cord revisited. Brain Pathol Zurich Switz. 2018 May;28(3):334–48.

32. Lucchinetti CF, Guo Y, Popescu BFG, Fujihara K, Itoyama Y, Misu T. The pathology of an autoimmune astrocytopathy: lessons learned from neuromyelitis optica. Brain Pathol Zurich Switz. 2014 Jan;24(1):83–97.

33. Bradl M, Reindl M, Lassmann H. Mechanisms for lesion localization in neuromyelitis optica spectrum disorders. Curr Opin Neurol. 2018 Jun;31(3):325–33.

34. Borisow N, Mori M, Kuwabara S, Scheel M, Paul F. Diagnosis and Treatment of NMO Spectrum Disorder and MOG-Encephalomyelitis. Front Neurol. 2018;9:888.

35. Narayan R, Simpson A, Fritsche K, Salama S, Pardo S, Mealy M, Paul F, Levy M. MOG antibody disease: A review of MOG antibody seropositive neuromyelitis optica spectrum disorder. Mult Scler Relat Disord. 2018 Oct 1;25:66–72.

36. Waters P, Woodhall M, O'Connor KC, Reindl M, Lang B, Sato DK, Juryńczyk M, Tackley G, Rocha J, Takahashi T, Misu T, Nakashima I, Palace J, Fujihara K, Leite MI, Vincent A. MOG cell-based assay detects non-MS patients with inflammatory neurologic disease. Neurol Neuroimmunol Neuroinflammation. 2015 Jun;2(3):e89.

37. Sinnecker T, Dörr J, Pfueller CF, Harms L, Ruprecht K, Jarius S, Brück W, Niendorf T, Wuerfel J, Paul F. Distinct lesion morphology at 7-T MRI differentiates neuromyelitis optica from multiple sclerosis. Neurology. 2012 Aug 14;79(7):708–14.

38. D'Souza M, Yaldizli Ö, John R, Vogt DR, Papadopoulou A, Lucassen E, Menegola M, Andelova M, Dahlke F, Schnyder F, Kappos L. Neurostatus e-Scoring improves consistency of Expanded Disability Status Scale assessments: A proof of concept study. Mult Scler Houndmills Basingstoke Engl. 2017 Apr;23(4):597–603.

39. Chakravarty MM, Steadman P, van Eede MC, Calcott RD, Gu V, Shaw P, Raznahan A, Collins DL, Lerch JP. Performing label-fusion-based segmentation using multiple automatically generated templates. Hum Brain Mapp. 2013 Oct;34(10):2635–54.

40. Purves D, Augustine GJ, Fitzpatrick D, Katz LC, LaMantia A-S, McNamara JO, Williams SM. The Major Afferent Pathway for Mechanosensory Information: The Dorsal Column-Medial Lemniscus System. Neurosci 2nd Ed [Internet]. 2001 [cited 2020 Mar 18]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK11142/

2. Affidavit/Statutory Declaration

"I, Claudia Chien, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic **Spinal cord atrophy measured from cerebral T1-weighted MRI: applications in clinical investigations of neuromyelitis optica spectrum disorders**, independently and without the support of third parties, and that I used no other sources and aids than those stated.
All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I am aware of the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice and that I commit to comply with these regulations.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

Date Signature

3. Declaration of contribution to the publications

Claudia Chien contributed the following to the below listed publications:

Publication 1: **Chien C**, Brandt AU, Schmidt F, Bellmann-Strobl J, Ruprecht K, Paul F, Scheel M. MRI-Based Methods for Spinal Cord Atrophy Evaluation: A Comparison of Cervical Cord Cross-Sectional Area, Cervical Cord Volume, and Full Spinal Cord Volume in Patients with Aquaporin-4 Antibody Seropositive Neuromyelitis Optica Spectrum Disorders. Am J Neuroradiol. 2018 Jul;39(7):1362-8.

Contribution (in detail): Claudia Chien performed the MRI pre- and post-processing, segmentations, statistical analysis, as well as produced figures, graphs, tables and written work for this publication. She also worked on the study design, interpretation of results, and wrote revisions prior to publication with the help of M.S., A.U.B, and F.P.
All co-authors aided in recruiting patients and healthy participants, collecting clinical and/or MRI data used for this study, the interpretation of results, gave suggestions for statistical/written edits, study design, and financial/technical support of data collection and analysis.

Publication 2: **Chien C,** Scheel M, Schmitz-Hübsch T, Borisow N, Ruprecht K, Bellmann-Strobl J, Paul F, Brandt AU. Spinal cord lesions and atrophy in NMOSD with AQP4-IgG and MOG-IgG associated autoimmunity. Mult Scler Houndmills Basingstoke Engl. 2019 Dec;25(14):1926–36.

Contribution (in detail): Claudia Chien performed the MRI pre- and post-processing, segmentations, lesion and clinical data evaluation, statistical analysis, produced figures, graphs, tables, and written work for this publication. She also worked on the study design, interpretation of results, and wrote revisions prior to publication with the help of F.P and A.U.B.
All co-authors made contributions to this manuscript by recruiting patients and healthy participants, collecting clinical data used for this study, also gave their interpretation of results, suggestions for statistical/written edits, study design, and financial/technical support of data collection and analysis.

Publication 3: Papadopoulou A, Oertel FC, Gaetano L, Kuchling J, Zimmermann H, **Chien C,** Siebert N, Asseyer S, Bellmann-Strobl J, Ruprecht K, Chakravarty MM, Scheel M, Magon S, Wuerfel J, Paul F, Brandt AU. Attack-related damage of thalamic nuclei in neuromyelitis optica spectrum disorders. J Neurol Neurosurg Psychiatry. 2019 Oct 1;90(10):1156–64.

Contribution (in detail): All MRI pre- and post-processing for MUCCA segmentations for this publication were performed by Claudia Chien, leading to the construction of Figure 3C and the results under “Volume of the VPN and myelitis”.

_____
Signature, date and stamp of supervising university professor / lecturer

_____
Signature of doctoral candidate

4. Print versions of the selected publications

4.1 Publication 1

Chien C, Brandt AU, Schmidt F, Bellmann-Strobl J, Ruprecht K, Paul F, Scheel M. MRI-Based Methods for Spinal Cord Atrophy Evaluation: A Comparison of Cervical Cord Cross-sectional Area, Cervical Cord Volume, and Full Spinal Cord Volume in Patients with Aquaporin-4 Antibody Seropositive Neuromyelitis Optica Spectrum Disorders, *Am J Neuroradiol*. 2018 Jul;39(7):1362-8.

http://dx.doi.org/10.3174/ajnr.A5665

4.2 Publication 2

Chien C, Scheel M, Schmitz-Hübsch T, Borisow N, Ruprecht K, Bellmann-Strobl J, Paul F, Brandt AU. Spinal cord lesions and atrophy in NMOSD with AQP4-IgG and MOG-IgG associated autoimmunity, *Mult Scler Houndmills Basingstoke Engl.* 2019 Dec;25(14):1926–36.

https://doi.org/10.1177/1352458518815596

4.3 Publication 3

Papadopoulou A, Oertel FC, Gaetano L, Kuchling J, Zimmermann H, **Chien C**, Siebert N, Asseyer S, Bellmann-Strobl J, Ruprecht K, Chakravarty MM, Scheel M, Magon S, Wuerfel J, Paul F, Brandt AU. Attack-related damage of thalamic nuclei in neuromyelitis optica spectrum disorders*, J Neurol Neurosurg Psychiatry*. 2019 Oct 1;90(10):1156–64.

http://dx.doi.org/10.1136/jnnp-2018-320249

5. Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

My curriculum vitae is not published in the electronic version of my dissertation for data protection reasons.

6. Complete List of Publications

6.1 Peer-reviewed original research articles

1. Juenger V, Cooper G, **Chien C**, Chikermane M, Oertel FC, Zimmermann H, Ruprecht K, Jarius S, Siebert N, Kuchling J, Papadopoulou A, Asseyer S, Bellmann-Strobl J, Paul F, Brandt AU, Scheel M. Optic chiasm measurements may be useful markers of anterior optic pathway degeneration in neuromyelitis optica spectrum disorders. Eur Radiol [Internet]. 2020 Apr 26 [cited 2020 May 18]; Available from: https://doi.org/10.1007/s00330-020-06859-w.
   - Impact Factor (2018): 3.962

2. Albert C, Mikolajczak J, Liekfeld A, Piper SK, Scheel M, Zimmermann HG, Nowak C, Dörr J, Bellmann-Strobl J, **Chien C**, Brandt AU, Paul F, Hoffmann O. Fingolimod after a first unilateral episode of acute optic neuritis (MOVING) – preliminary results from a randomized, rater-blind, active-controlled, phase 2 trial. *BMC Neurol*. 2020 Mar 3;20(1):75.
   - Impact Factor (2018): 2.233

3. **Chien C**, Juenger V, Scheel M, Brandt AU, Paul F. Considerations for Mean Upper Cervical Cord Area Implementation in a Longitudinal MRI Setting: Methods, Interrater Reliability, and MRI Quality Control. *AJNR Am J Neuroradiol*. 2020 Feb;41(2):343–50.
   - Impact Factor (2018): 3.256

4. **Chien C,** Oertel FC, Siebert N, Zimmermann H, Asseyer S, Kuchling J, Scheel M, Ruprecht K, Bellmann-Strobl J, Paul F, Brandt AU. Imaging markers of disability in aquaporin-4 immunoglobulin G seropositive neuromyelitis optica: a graph theory study. *Brain Commun* [Internet]. 2019 Jan 1 [cited 2020 Mar 20];1(1). Available from: https://academic.oup.com/braincomms/article/1/1/fcz026/5588407.
   - Impact Factor (2018): Not yet available (new open access journal).

5. Papadopoulou A, Oertel FC, Gaetano L, Kuchling J, Zimmermann H, **Chien C**, Siebert N, Asseyer S, Bellmann-Strobl J, Ruprecht K, Chakravarty MM, Scheel M, Magon S, Wuerfel J, Paul F, Brandt AU. Attack-related damage of thalamic nuclei in neuromyelitis optica spectrum disorders. *J Neurol Neurosurg Psychiatry*. 2019 Oct 1;90(10):1156–64.
   - Impact Factor (2018): 8.272

6. Cooper G, Finke C, **Chien C**, Brandt AU, Asseyer S, Ruprecht K, Bellmann-Strobl J, Paul F, Scheel M. Standardization of T1w/T2w Ratio Improves Detection of Tissue Damage in Multiple Sclerosis. *Front Neurol* [Internet]. 2019 Apr 9 [cited 2019 May 13];10. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6465519/
   - Impact Factor (2018): 2.635

7. Ciccarelli O, Cohen JA, Reingold SC, Weinshenker BG, Amato MP, Banwell B, Barkhof F, Bebo B, Becher B, Bethoux F, Brandt A, Brownlee W, Calabresi P, Chatway J, **Chien C**, Chitnis T, Comi G, Correale J, De Sèze J, De Stefano N, Fazekas F, Flanagan E, Freedman M, Fujihara K, Galetta S, Goldman M, Greenberg B, Hartung HP, Hemmer B, Henning A, Izbudak I, Kappos L, Lassmann H, Laule C, Levy M, Lublin F, Lucchinetti C, Lukas C, Marrie RA, Miller A, Miller D, Montalban X, Mowry E, Ourselin S, Paul F, Pelletier D, Ranjeva JP, Reich D, Rocca MA, Rovira A, Schlaerger R, Soelberg Sorensen P, Sormani M, Stuve O, Thompson A, Tintoré M, Traboulsee A, Trapp B, Trojano M, Uitdehaag B, Vukusic S, Waubant E, Wheeler-Kingshott CG, Xu J. Spinal cord involvement in multiple sclerosis and neuromyelitis optica spectrum disorders. *Lancet Neurol*. 2019 Feb;18(2):185–97.
   - Impact Factor (2018): 28.755

8. **Chien C**, Scheel M, Schmitz-Hübsch T, Borisow N, Ruprecht K, Bellmann-Strobl J, Paul F, Brandt AU. Spinal cord lesions and atrophy in NMOSD with AQP4-IgG and MOG-IgG associated autoimmunity. *Mult Scler Houndmills Basingstoke Engl*. 2019 Dec;25(14):1926–36.
   - Impact Factor (2018): 5.649

9. Asseyer S, Schmidt F, **Chien C**, Scheel M, Ruprecht K, Bellmann-Strobl J, Brandt AU, Paul F. Pain in AQP4-IgG-positive and MOG-IgG-positive neuromyelitis optica spectrum disorders. *Mult Scler J - Exp Transl Clin.* 2018 Sep;4(3):2055217318796684.
   - Impact Factor (2018): Not yet available (new open access journal).

10. Akens MK, **Chien C**, Katchky RN, Kreder HJ, Finkelstein J, Whyne CM. The impact of thermal cycling on Staphylococcus aureus biofilm growth on stainless steel and titanium orthopaedic plates. *BMC Musculoskelet Disord.* 2018 Jul 27;19(1):260.
   - Impact Factor (2018): 2.002

11. **Chien C**, Brandt AU, Schmidt F, Bellmann-Strobl J, Ruprecht K, Paul F, Scheel M. MRI-Based Methods for Spinal Cord Atrophy Evaluation: A Comparison of Cervical Cord Cross-Sectional Area, Cervical Cord Volume, and Full Spinal Cord Volume in Patients with Aquaporin-4 Antibody Seropositive Neuromyelitis Optica Spectrum Disorders. *AJNR Am J Neuroradiol.* 2018 Jul;39(7):1362–8.
   - Impact Factor (2018): 3.256

12. Oertel FC, Kuchling J, Zimmermann H, **Chien C**, Schmidt F, Knier B, Bellmann-Strobl J, Korn T, Scheel M, Klistorner A, Ruprecht K, Paul F, Brandt AU. Microstructural visual system changes in AQP4-antibody-seropositive NMOSD. *Neurol Neuroimmunol Neuroinflammation.* 2017 May;4(3):e334.
   - Impact Factor (2018): 7.353

13. Schlemm L, **Chien C**, Bellmann-Strobl J, Dörr J, Wuerfel J, Brandt AU, Paul F, Scheel M. Gadopentetate but not gadobutrol accumulates in the dentate nucleus of multiple sclerosis patients. *Mult Scler Houndmills Basingstoke Engl*. 2017 Jun;23(7):963–72.
   - Impact Factor (2018): 5.649

14. Bisland SK, **Chien C**, Wilson BC, Burch S. Pre-clinical in vitro and in vivo studies to examine the potential use of photodynamic therapy in the treatment of osteomyelitis. *Photochem Photobiol Sci Off J Eur Photochem Assoc Eur Soc Photobiol*. 2006 Jan;5(1):31–8.
   - Impact Factor (2018): 2.408

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