Aus dem Institut für Neuropathologie
(Prof. Dr. med. W. Brück)
der Medizinischen Fakultät der Universität Göttingen

Apheresis therapy in immunopathologically classified multiple sclerosis patients

INAUGURAL-DISSERTATION

zur Erlangung des Doktorgrades
der Medizinischen Fakultät der
Georg-August-Universität zu Göttingen

vorgelegt von

Lidia Stork (geb. Sviderskaya)

aus

Krasnojarsk/Russland

Göttingen 2018

Dekan:	Prof. Dr. rer. nat. H. K. Kroemer

Betreuungsausschuss

Betreuer/in	Prof. Dr. med. I. Metz
Ko-Betreuer/in:	Prof. Dr. med. K. Hein

Prüfungskommission

Referent/in	Prof. Dr. med. I. Metz
Ko-Referent/in:	Prof. Dr. med. M. Koziolek
Drittreferent/in:	Prof. Dr. med. P. Huppke

Datum der mündlichen Prüfung: 05.02.2020

I hereby declare that I have written my doctoral thesis entitled "Apheresis therapy in immunopathologically classified multiple sclerosis patients" independently and with no other sources and aids than those quoted.

Göttingen, …… ……

(Unterschrift)

Contents

List of Figures

List of Tables

Abbreviations

ADEM	Acute disseminated encephalomyelitis
AQP4	Aquaporin 4
C3	Complement 3
C9neo	Complement 9 neo
CD	Cluster of differentiation
CD5L	CD5 antigen-like protein
CIDP	Chronic inflammatory demyelinating polyneuropathy
CIS	Single clinical episode
Cl	Confidential interval
CNPase	2'3'-cyclic nucleotide 3'phosphodiesterase
CNS	Central nervous system
CSF	Cerebral spinal fluid
DMD	Disease-modifying drug
EDSS	Expanded disability status scale
FLAIR	Fluid-attenuated invention recovery imaging
Gd	Gadolinium
HDCS	High-dose corticosteroids
HLA	Human leukocyte antigen
IA	Immunoadsorption
ICAM-1	Intracellular adhesion molecule 1
IgG	Immunoglobulin G
IL8	Interleukin 8
LDCS	Low-dose corticosteroids
LogOR	Logarithmic odds ratio
MAG	Myelin-associated glycoprotein
MBP	Myelin basic protein
MOG	Myelin oligodendrocyte glycoprotein

MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NMDA	N-methyl-D-aspartate
NMO	Neuromyelitis optica
NMOSD	Neuromyelitis optica spectrum disorders
OCBs	Oligoclonal bands
PLEX	Therapeutic plasma exchange
PLP	Proteolipid protein
PPMS	Primary progressive multiple sclerosis
RRMS	Relapsing-remitting multiple sclerosis
SE	Standard error
SPMS	Secondary progressive multiple sclerosis
T2W	T2-weighted MRI image
T1W+Gd	T1-weighted MRI image with gadolinium enhancement
Th1/Th2	T helpers ½
TNF-α	Tumor necrosis factor α
TTP	Thrombotic thrombocytopenic purpura
VCAM-1	Vascular cell adhesion molecule 1

1 Introduction

Multiple Sclerosis (MS) is chronic inflammatory demyelinating disease of the central nervous system that affects more than two million young adults worldwide (Weinshenker 1996). Females are affected more often than males (2,5:1)(Koch-Henriksen and Sorensen 2010; Niedziela et al. 2014), and the disease usually begins in the second or third decade of life. Different disease courses can be distinguished. Most of the patients have a relapsing–remitting disease course (RRMS), with symptom exacerbation (relapse) over hours to days followed by a relapse-free period. Symptoms may regress spontaneously or in response to anti-inflammatory corticosteroid therapy. Neurological disability may accumulate during the disease course. In later disease stages disability may progress without clinical relapses, indicating secondary progressive multiples sclerosis (SPMS). One fifth of the MS patients manifest with a progressive disease course from the beginning (primary progressive MS, PPMS), with a similar incidence among men and women. Expanded disability status scale (EDSS) is used to measure neurological disability in MS. This scale refers to neurological findings and ranges from 0 (no impairment) to 10 (death from MS)(Amato and Ponziani 1999).

Magnetic resonance imaging (MRI) of the brain serves for diagnosis and differential diagnosis of MS. In MS, it typically shows hyperintense lesions on a T2-weighted (T2W) or on fluid attenuation inversion recovery (FLAIR) imaging techniques located periventricularly, so-called Dawson's fingers (Tillema et al. 2013). Intravenous administration of the contrast agent gadolinium (Gd) leads to an accumulation of Gd within the lesions and indicates blood-brain barrier leakage. In these regions, inflammatory cells invade the CNS and inflammatory infiltrates are found. This enhancement is typically seen during the first 4-6 weeks after lesion formation and helps to distinguish between old and new demyelinating lesion on MRI. (Cotton et al. 2003).

Dissemination of lesions in space and time must be present to fulfill the diagnostic criteria of MS (Polman et al. 2011). A clinical history of two or more attacks in a young adult with typical neurological symptoms such as visual problems, paresis or ataxia indicates a diagnosis of MS. In case of a single clinical exacerbation, additional MRI should support the diagnosis. MRI evidences of dissemination in space (involvement of at least two of the following regions: periventricular, cortical or juxtacortical, infratentorial, optic nerve and spinal cord) and dissemination in time (new T2 lesion/s or simultaneous presence of contrast-enhancing and non-contrast-enhancing lesions) are required for MS diagnosis (Filippi et al. 2016). The presence of oligoclonal bands (OCBs) and elevated immunoglobulin G (IgG) in the cerebral spinal fluid (CSF) support the diagnosis of MS, but are not specific for it (Stangel et al. 2013).

1.1 Pathogenesis and histopathological heterogeneity of multiple sclerosis

Demyelination in MS is generally believed to be caused by pathological immune responses to CNS self-antigens; however, the exact mechanism of MS development is unknown. T cells, B cells and probably autoantibodies are important factors contributing to MS development (Sospedra et al. 2005). Environmental factors such as viral infections (especially Epstein-Barr virus), metabolic stress, obesity, smoking and vitamin D deficiency in genetically susceptible people may facilitate migration of myelin-specific, auto-reactive immune cells across the blood-brain barrier, leading to demyelination, axonal destruction and subsequent neurological disability (Shaygannejad et al. 2016). Inflammatory demyelinating plaques are characterized by a confluent myelin loss with relative preservation of axons and an astrogliosis. Histopathological studies indicate the role of the adaptive immune system in disease development, showing that MS lesions contain inflammatory cells with CD8+ cytotoxic T cells dominating over CD4+ T helper cells, as well as numerous macrophages. B cells and plasma cells are present in variable numbers. Inflammatory cells are typically located around vessels, but also diffusely infiltrate the parenchyma. The composition of the immune cell infiltrate and the presence of myelin degradation products within the macrophages depend on the stage of the lesional activity (Bruck et al. 1995).

Histological findings also show the heterogeneity of MS lesions and suggest that more than one pathogenic mechanism contributes to disease development. Early active demyelinating lesions can be classified histopathologically into three immunopathological patterns (patterns I-III), suggesting different pathogenic mechanisms that lead to lesion development (Lucchinetti et al. 2000). Within a single patient, the immunopathological pattern does not change during the disease course (Metz et al. 2014). Patterns I and II share similar features of demyelination. In both patterns, T lymphocytes and macrophages dominate the lesions. Plaques typically show sharply demarcated lesion borders and in early lesions stages, remyelination can often be observed. The only differences between these two patterns are immunoglobulin and complement deposits found inside macrophages in pattern II. In conclusion, an antibody-mediated mechanism of lesion development may be assumed in pattern II. In contrast, pattern III lesions are characterized by the presence of apoptotic oligodendrocytes at the lesion edge and a preferential loss of myelin-associated glycoprotein (MAG). MAG is a myelin protein located in distal oligodendrocyte processes and its loss is considered to be a marker of metabolically stressed oligodendrocytes. Thus, changes observed in pattern III lesions possibly reflect primary oligodendrocytic damage (Aboul-Enein et al. 2003).

1.2 Apheresis therapy in multiple sclerosis

Multiple sclerosis cannot be cured, but various drugs are available that can modify the disease course (DMDs) and lead to a milder disability. Different treatment approaches are used for the therapy during the relapse (acute exacerbations of the disease) and for long-term therapy. Treatment of the relapse aims to suppress the acute episode of inflammatory demyelination in the brain. High-dose glucocorticosteroids (HDCS) are primarily recommended for this purpose. In contrast, the long-term therapy is needed to prevent the development of new relapses and progression of the disease. For that, several immunomodulatory drugs were approved for various MS disease courses.

Therapeutic plasma exchange (PLEX) and immunoadsorption (IA) are apheresis techniques, which are used in MS patients and are recommended by US and European neurologists as a second line treatment for MS relapses in case of insufficient response to HDCS (Schwartz et al. 2013; Bevan et al.2015).

The main principle of these methods is to purify the serum of patients from disease-causing agents such as antibodies/auto-antibodies, immune complexes and cytokines (McLeod 2010; Okafor et al. 2010; Williams und Balogun 2014). During PLEX the serum of the patients is replaced with a serum replacement solution, whereas with IA the serum passes through the absorber column and is then returned to the blood circulation.

Apheresis is a second line treatment, and treatment success for the individual patient is not predictable. Several retrospective and prospective studies have shown that the efficiency of both PLEX and IA for MS relapses is comparable and varies from 40 - 90% (Weinshenker et al. 1999, Moldenhauer et al. 2005; Magana et al. 2011; Koziolek et al. 2013; Ehler et al. 2015). Male sex, early initiation of apheresis treatment and the presence of ring-like, contrast-enhancing lesions on MRI are associated with a favorable outcome after PLEX/IA treatment (Keegan et al. 2002; Llufriu et al. 2009; Magana et al. 2011). In 2005, Keegan et al. suggested that humoral features could explain the variability in the apheresis response in the MS population. Their study proposed that apheresis therapies may be a therapeutic option for pattern II patients, which are characterized by immunoglobulin deposition and complement activation within lesions (Keegan et al. 2005).

The aim of the present study was to identify clinical, demographical and histopathological parameters that could predict PLEX/IA response in steroid-resistant MS relapses.

2 Patients and methods

This study was approved by the ethics committee of the University Medical Center Göttingen (#19/09/10). The study cohort was recruited from our German brain biopsy databank, which includes 774 patients nationwide with histologically proven inflammatory demyelination consistent with MS. Among those, 386 cases showed an early active inflammatory demyelination, classifiable into immunopathological patterns. Sixty-nine patients who received apheresis therapy due to a steroid-resistant relapse and who had sufficient clinical and radiological information were included in the study. Clinical information was obtained from a medical record review (n=69). Treatment response was assessed retrospectively and blinded to the histopathologically defined immunopatterns.

2.1 Clinical and radiological follow-up

Diagnosis at the time of PLEX/IA was made based on published criteria for MS (Polman et al. 2011). The clinical course was classified as single clinical episode (CIS), relapsing-remitting or secondary-progressive at the time of treatment (Lublin 2014). Index attack was defined as the relapse leading to apheresis therapy. The following neurological systems were evaluated to assess which deficits occurred with the index attack: consciousness (somnolence, sopor, coma), cerebral (e.g. aphasia, apraxia), cognitive (memory dysfunction, disorientation), motor, brainstem or cranial nerves, cerebellar, sensory, and bladder/bowel dysfunction. Only new or worsening symptoms occurring with the index attack that influenced the EDSS score or significantly impacted function were considered.

Clinical information was extracted from the medical charts before apheresis, including the presence of deep tendon reflexes, treatment of the index attack with high/low dose corticosteroids (HDCS/LDCS), therapy with DMDs within 3 months before PLEX/IA initiation, as well as the CSF cell number, IgG index and presence of OCBs. MRI was evaluated for the presence of ring-like, Gd+-enhancing lesions. Immunosuppressive/immunomodulatory medications within 30 days after apheresis application were noted as well.

Treatment response was evaluated based on three main outcome parameters. The primary outcome was functional changes in the neurological system affected during the relapse. For this we used a response evaluation that was published previously (Weinshenker 1999). According to this score, none or mild subjective changes in the affected neurological system were interpreted as no response, moderate or important gain in neurological status were considered to be a treatment response. MRI and EDSS changes were analyzed as secondary outcome parameters. Lesions were investigated using T2W images as well as Gd-enhanced T1-weighted images (T1W+Gd). MRI improvement was defined by lesion

shrinkage and/or reduction in gadolinium enhancement. EDSS scores were evaluated at three time points: 1) last EDSS at relapse-free period before index attack (baseline EDSS), 2) highest EDSS of index attack before PLEX/IA treatment (EDSS relapse) and 3) EDSS within one month after apheresis therapy (EDSS 1 month). EDSS treatment response one month after apheresis therapy was defined as a reduction in the EDSS score ≥0.5 points in patients with an EDSS score ≥6.0 at the time of index attack, or a reduction ≥1.0 in patients with an EDSS ≤ 5.5 before treatment was started ("Guidline on clinical inverstigation of medical products for the treatment of multiple sclerosis" 2015).

2.2 Histopathology and classification of the lesions

Histological classification of lesions was performed as described in previous publications (Lucchinetti et al. 2000; Metz et al. 2014). For the classification of lesions, first the demyelinating activity was determined based on published criteria with early active demyelinating lesions containing myelin-laden macrophages immunoreactive both for minor and major myelin proteins (Bruck et al. 1995). Those lesions were subsequently classified into one of the immunopathological patterns I-III (Lucchinetti et al. 2000). Histopathological analysis was performed blinded to PLEX/IA response.

Tissue sections were analyzed using an Olympus BX41 microscope (Olympus Optical Co, Ltd., Hamburg, Germany). Figures were prepared in CorelDraw X3®, version 13.

2.3 Statistical analyses

The statistical analysis was performed in cooperation with the Institute of Medical Statistics of the University Medical Center Goettingen (David Ellenberger, Prof. Tim Friede, Prof. Tim Beissbarth). Descriptive statistics are given for the cohort as a whole and by immunopattern strata. These include: frequencies for the categorical outcomes sex, disease course, HDCS and DMD treatment, medians (and minimum and maximum) for the ordinal outcome EDSS as well as mean (and standard deviation) for the metrical outcomes age, PLEX/IA delay (time interval between index attack onset and initiation of PLEX/IA treatment), and disease duration (time from first neurological symptoms ever to initiation of PLEX/IA treatment). Comparisons for global group differences were made using Fisher's exact test and the Kruskal-Wallis test or the one-way analysis of variance. To adjust for relevant covariates the effect of the immunopatterns on treatment response in the primary and secondary outcomes were analyzed using logistic regression models. Firth correction was used to avoid model fitting problems due to very low response rates in some subgroups. Univariate and multivariate effect measures along with penalized likelihood profiles-based 95% confidence intervals as well as predicted probabilities for various subgroups are given. Selection of relevant covariates was initially done using lasso (least absolute shrinkage and selection operator) and subsequently by backward variable

selection, eliminating statistically less informative variables to avoid overfitting. Only variables that significantly differed between the IP strata were kept permanently in the model unless major collinearities appeared. When addressing longitudinal measurements of serial PLEX/IA sessions within single patients, generalized estimation equations with a compound symmetry covariance structure were used to estimate whether a response is predictive for future responses while adjusting for relevant covariates. Statistical analyses were carried out with SAS 9.4 and R (Version 3.1.2). In general, two-sided p-values smaller than or equal to 5% were regarded as statistically significant.

3 Results and Discussion

3.1 Patient demographics and baseline clinical features

Apheresis therapies are invasive treatment approaches and are associated with several complications, which in some rare cases can be life-threatening. Among the severe side effects are an arterial blood pressure fall, electrolyte imbalance with arrhythmias, hemolysis, but also anxiety, vomiting, paresthesias and allergic reactions have been described (Szczeklik et al. 2013). On the other hand, 40-90% of patients improve clinically after apheresis therapies and thus benefit from this therapeutic option. Therefore, predictors for a therapy success would be helpful for decision making in clinical practice.

In our study we assessed retrospectively different clinical and histological parameters in 69 patients with histopathologically verified and classified inflammatory demyelinating disease compatible with MS, with the aim to find predictors of a PLEX/IA response. Demographical, clinical and histological characteristics are summarized in Table 1.

Table 1: Demographic, clinical and histological characteristics of PLEX/IA cohort at the time of apheresis treatment

Demographical, clinical and histological parameters	
Sample size	n = 69
Age: mean(sd)	36.6 (13.3)
Proportion of females (%)	46/69 (66.7%)
Disease course: Single clinical episode (%)	28/69 (40.6%)
Disease course: RR (%)	36/69 (52.2%)
Disease course: SP (%)	5/69 (7.2%)
Disease duration (years): median (min, max)	0.2 (0.0,18.0)
EDSS baseline: median (min, max)	1.0 (0.0,8.5)
EDSS at index attack: median (min, max)	6.0 (2.0,9.5)
PLEX/IA delay (days): mean(sd)	25.4 (20.4)
Therapy with HDCS before PLEX/IA (%)	63/69 (91.3%)
Therapy with DMD within 3 months before PLEX/IA (%)	17/67 (25.4%)

Immunopathological pattern I	16/69
Immunopathological pattern II	40/69
Immunopathological pattern III	13/69

Abbreviations: RR: relapsing remitting; SP: secondary progressive; EDSS: expanded disability status scale; PLEX: plasma exchange; IA: immunoadsorption; HDCS: high dose of corticosteroids; DMD: disease modifying drugs, sd: standard deviation, min: minimum, max: maximum.

At the time of apheresis therapy, more than two-thirds of the patients (74%; n=51) had clinically definite MS according to the 2011 McDonald criteria. Ten patients had a single clinical episode (one relapse), but fulfilled the McDonald criteria for MS diagnosis. About one fifth of the patients (16/69, 23%) showed histological characteristics of early active inflammatory demyelinating lesions consistent with immunopathological pattern I. More than a half of the patients (40/69, 58%) were diagnosed histopathologically with immunopathological pattern II and 19% (13/69) of the patients showed histopathological features of pattern III.

The median number of PLEX exchanges/IAs was 5.0, the therapeutic regimen being determined by the treating physician. IA was performed in 15% of patients (n=10). Three of those patients had combined PLEX and IA treatments. Most of the patients received high dose corticosteroids (91%; n=63) and one fourth (25%; n=17) DMDs within 3 months before PLEX/IA application.

3.2 Response to apheresis treatment

Treatment response was evaluated within 30 days after the PLEX/IA application and was based on the main outcome parameters: functional improvement, MRI and EDSS response (see Methods). Thirty-nine percent of the patients showed functional (27/69) and/or MRI (18/46) improvement after PLEX/IA treatment. This percentage is consistent with published data of 40-90% (Weinshenker et al. 1999, Magana et al. 2011). EDSS response rate was 28% (19/67) and thus lower as compared to the functional response observed (Figure 1). Due to EDSS insensitivity to the changes of the function of upper extremities or cognition, six patients with functionally important improvement did not showed changes in the EDSS (Meyer-Moock S et al. 2014).

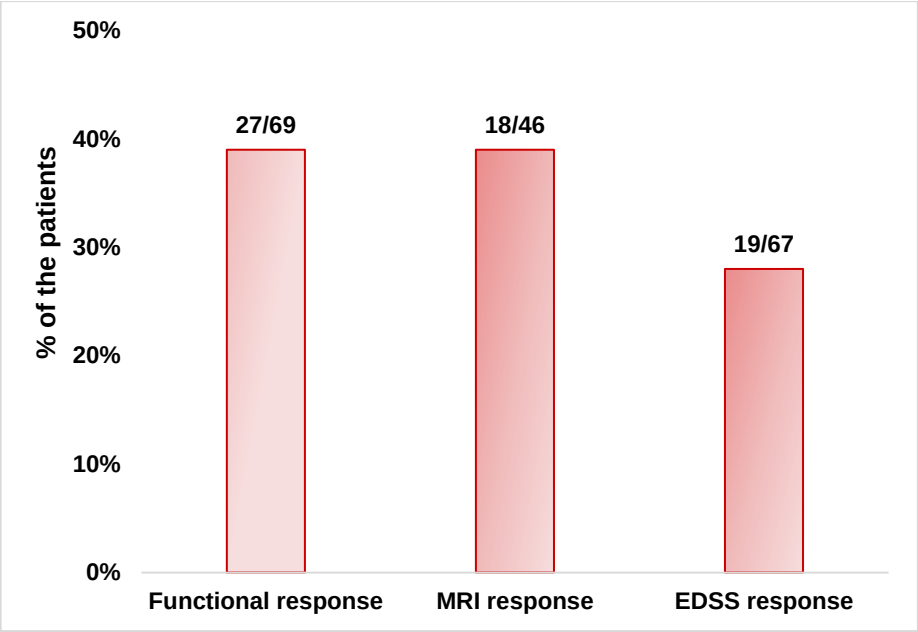

Figure 1: Functional, MRI and EDSS response to the apheresis therapy

The percentage of the patients with functional (moderate or marked functional improvement), MRI (lesions that were shrunk and/or showed less contrast enhancement) and EDSS (EDSS improvement ≥ 0.5 in patients with EDSS score ≥ 6.0 and an EDSS improvement ≥ 1.0 in patients with EDSS score ≥ 5.5) response.

However only 3% (2/27) of the patients showed a complete recovery after the treatment. Most patients still had residual deficits in the system that were affected during the index attack, and 84% presented with multifocal neurological deficits involving more than one functional system. Index attack symptoms leading to PLEX/IA treatment are shown in Figure 2. Motor dysfunction (75% of patients) and brainstem involvement (57%) were the most frequently targeted neurological systems (Figure 2).

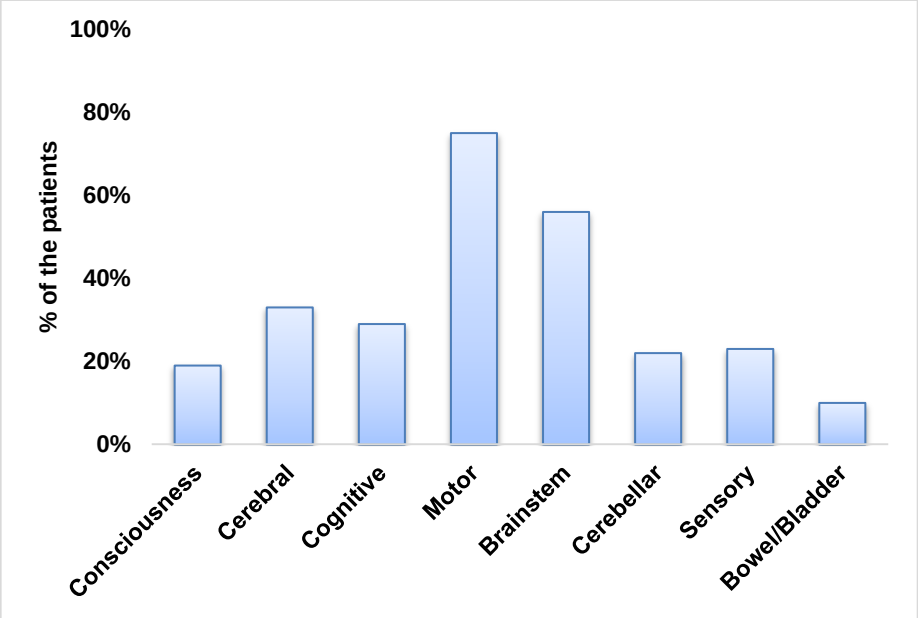

Figure 2: Affected neurological systems during index attack

Neurological systems affected. Data are presented as the percentage of patients with affection of the specified functional system in relation to all patients. Most of the patients presented with a polysymptomatic index attack and thus have more than one neurological system affected.

A clinical improvement could be observed in most functional systems (Figure 3). The highest response rate was evident for the functional system consciousness (42%, n=5/12) and the cerebellar system (43%, n=6/14), followed by the cognitive (30%, n=6/20) and motor systems (29% n= 14/49).

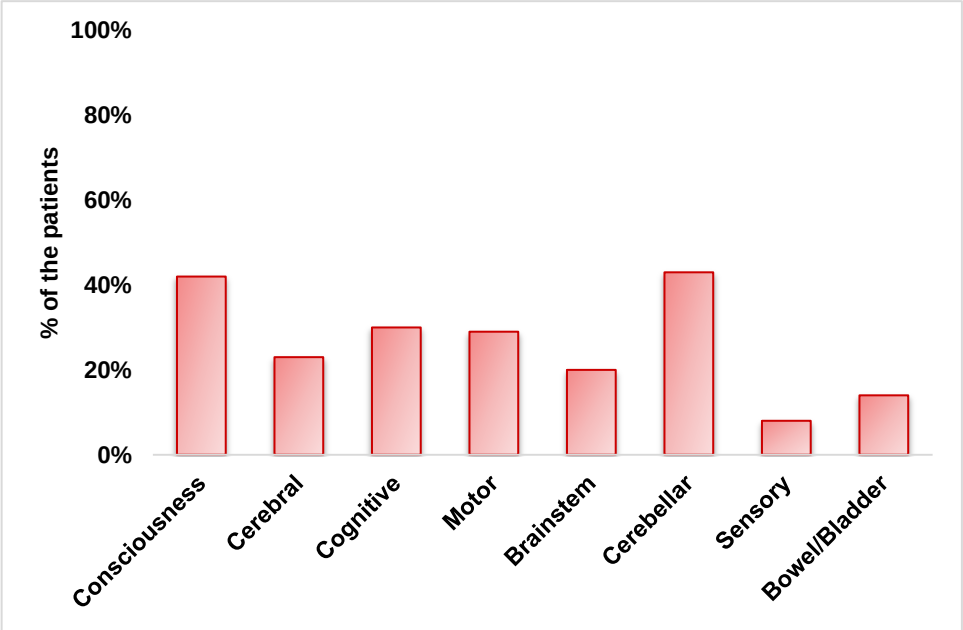

Figure 3: Functional response to apheresis therapy stratified to different neurological systems

Data are presented as the percentage of patients with improvement of the specified functional system in relation to all patients with this functional system affected. For some patients, clinical data were not sufficient to judge therapy response in single functional systems.

In summary, our data show similar clinical response rates to apheresis therapies as in previously published MS cohorts.

3.3 Predictors of the apheresis response.

Next we used multifactorial logistic regression analysis to identify demographical, clinical and histopathological parameters that could predict a PLEX/IA response. Previous studies reported that male sex, preserved reflexes, early initiation of the treatment and lower baseline EDSS were associated with better treatment outcome (Keegan et al. 2002; Llufriu et al., 2009; Ehler et al.). Additionally we analyzed index attack-related symptoms, disease severity, histopathological patterns, MRI parameters as well as CSF variables in univariate and multivariate logistic regressions (Figure 4).

Four parameters came out to be positive predictive factors during this analysis: These are immunopathological pattern I (logOR: 3.35, 95% CI: 0.57-8.59, p=0.014) and II (logOR:

5.61, 95% CI: 2.49-11.32, p<0.001), as well as application of the IA compared to PLEX (logOR: 3.26, 95% CI: 0.75-8.1, p=0.008) and, with a lower effect size, new cognitive deficits at the time of index attack. (logOR: 1.56, 95% CI: 0.03-4.37, p=0.046). In contrast, involvement of the brainstem and cranial nerves emerged as negative predictive factors (logOR: -1.43, 95% CI: -3.21 – -0.17, p=0.026) for an apheresis treatment response.

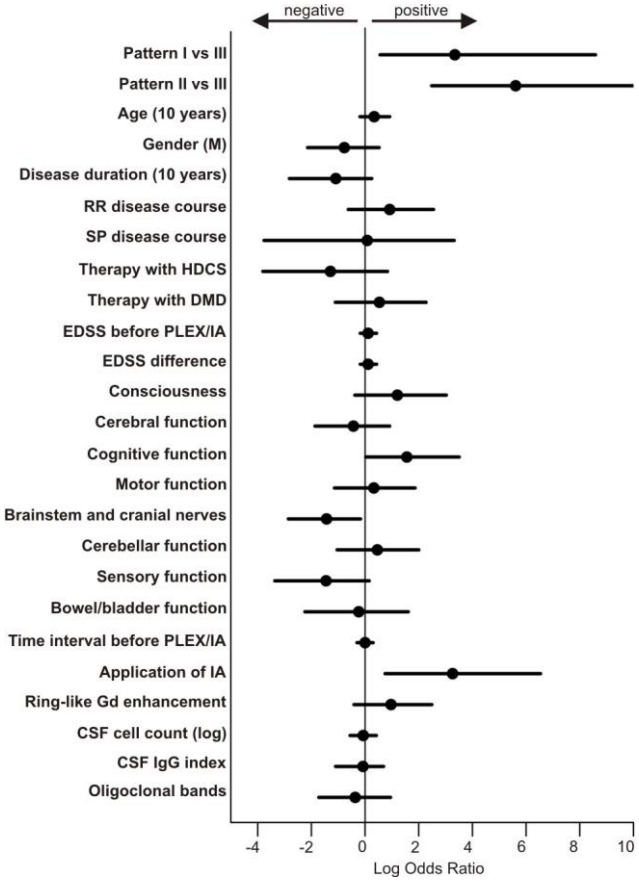

Figure 4: Logistic regression model of the PLEX/IA response

Effect estimates on functional response to apheresis therapy: The estimated log odds ratio of relevant covariates including penalized likelihood profiles-based 95% confidence intervals are given on whether patients experienced a moderate or marked functional improvement after PLEX/IA treatment. Covariates with a negative log odds ratio predict no therapy response, covariates with a positive log odds ratio predict therapy success. Covariate estimates are significant when the 95% confidence interval does not cross the log odd ratio 0. Estimates were obtained by multivariate logistic regression using Firth correction. Estimates indicate patterns I and II, affection of the cognitive system and therapy with immunoadsorption as covariables associated with a therapy success, with the pattern II showing the highest log odds ratio. Estimates suggest that brainstem affection is associated with a treatment failure. Multivariate (or more precisely, 'multivariable') adjustment included the following covariates: immunopattern, affection of the neurological systems brainstem or cognitive functions, therapy with immunoadsorption and disease duration as well as delay of PLEX/IA treatment. The covariable CSF cell count is shown logarithmized.

Abbreviations: RR disease course: relapsing–remitting disease course; SP disease course: secondary progressive disease course; HDCS: high-dose corticosteroids; DMD: disease-modifying drug; EDSS: expanded disability status scale; PLEX: plasma exchange; IA: immunoadsorption; CSF: cerebral spinal fluid; IgG: immunoglobulin G. Reproduced with permission from JAMA Neurology 2018, 75(4): 428-435. Copyright© (2018) American Medical Association. All rights reserved.

Estimating predicted probability we found out that the highest probability for the therapy response was in pattern II patients with no brainstem involvement who were treated with IA (99 %; Table 2), whereas the lowest probability of a therapy response was in a pattern III patient with brainstem and cranial nerve involvement (0%, Table 2). It should be noted that in peripheral subgroups, the predicted therapy response rate might be overfitted.

Table 2: Predicted probability of therapy response to apheresis treatments stratified by immunopathological patterns, brainstem involvement and affection of the cognitive functions at index attack

	IA		**IA**		**PLEX**		**PLEX**	
Pattern I	89% (49-98)	91% (54-99)	65% (23-92)	69% (26-94)	23% (8-53)	29% (10-59)	7% (1-26)	8% (2-30)
Pattern II	99% (83-100)	99% (84-100)	95% (64-99)	96% (68-100)	74% (54-88)	80% (51-94)	41% (24-60)	45% (31-61)
Pattern III	21% (1-82)	27% (2-85)	6% (0-47)	7% (0-54)	1% (0-19)	1% (0-21)	0% (0-6)	0% (0-7)
	Brainstem -	**Cognition +**	**Brainstem +**	**Cognition -**	**Brainstem -**	**Cognition +**	**Brainstem +**	**Cognition -**

The predicted percentage of patients responding to apheresis treatment and 90% confidential interval are given. In peripheral subgroups the predicted therapy response rate might be overfitted.
Abbreviations: IA: immunoadsorption, PLEX: plasma exchange, Brainstem - : Brainstem not affected at index attack, Brainstem +: Brainstem affected at index attack. Cognition -: Cognitive function not affected at index attack, Cognition +: Cognitive function affected at index attack. Reproduced with permission from JAMA Neurology 2018, 75(4): 428-435. Copyright© (2018) American Medical Association. All rights reserved.

The study of Keegan et al. analyzed the PLEX response with respect to histopathologically determined immunopathological patterns (Keegan et al. 2005). Of their 19 patients, only patients with pattern II pathology responded to PLEX treatment, but none of the patients with pattern I or III pathology. Thus, their study proposed that apheresis therapy is exclusively effective in pattern II patients, which are characterized by immunoglobulin deposition and complement activation within lesions. This is in line with the known efficacy of apheresis therapies in antibody-mediated diseases such as neuromyelitis optica (NMO) or myasthenia gravis (Gajdos et al. 2002; Kim et al. 2013; Yamada et al. 2015). In our study analysing a larger cohort of 69 patients, both immunopathological pattern I and II turned out to be positive predictive factors for an apheresis response (see also 3.4 for discussion).

In studies of myasthenia gravis, IA was associated with less severe side effects as compared to PLEX (Köhler et al. 2011). The previous studies in MS patients showed a significant clinical improvement after IA in 73-85% of MS patients compared to 40-70% after PLEX, indicating a similar efficacy (Koziolek et al. 2013, Schimrigk et al. 2016). Faissner et al, showed in a series of 48 patients that the combination of both PLEX and IA may be more effective than when only one of the treatments is applied alone (Faissner et al. 2016). However, controlled data comparing clinical efficacy of both methods in a clinical study are lacking. Our study was limited by the low number of patients treated with IA (n=10), so that further studies are necessary to explore whether IA may even have treatment effects superior to PLEX. Interestingly, IA not only removes antibodies but also other proteins such as complement factors, MBP, CD5L, transthyretin, serum amyloid P, that may be involved in MS pathogenesis (Koziolek et al. 2012).

Brainstem affection was observed here to be a negative predictive factor for therapy response. Prior studies, however, did not find such an association (Magana et al. 2011; Meca-Lallana et al. 2013). Some radiological studies have shown that patients with brainstem involvement had a worse prognosis, regardless of apheresis therapy (Trojano et al. 1995; Tintore et al. 2010). In this study, clinical involvement of the brainstem was not always accompanied by brainstem lesions on MRI. Thus, clinical brainstem involvement should be considered as a potential factor negatively influencing therapy response.

Previous studies reported that lesions with edema, mass effect and ring-like enhancement on MRI were associated with a beneficial therapy response to PLEX (Magana et al. 2011). The radiological appearance of a lesion reflects its pathological features. Ring-like enhancement is found in pattern I and pattern II lesions and correlates with a macrophage rim at the lesion border (Bruck et al. 2001). Therefore, ring-like enhancement on MRI could be helpful for predicting treatment response. Although a ring-like contrast enhancement was found significantly more often in pattern II than in pattern III patients (none of the pattern III patients showed ring enhancement), it was not independently associated with a favorable outcome. This may be due to the limited number of patients

with available MRIs and ring-like contrast enhancement in our study (n=16). Other previously reported clinical (early initiation of treatment, shorter disease duration, preserved deep tendon reflexes, baseline EDSS < 5.0) and demographic factors (male sex) associated with an apheresis therapy response could not be confirmed in the present study.

3.4 Mechanism of action of the apheresis therapy in three immunopathological patterns

Histological classification of the patients with early active demyelinating lesions turned out to be important for apheresis response prediction. The histopathological differences among the lesion are intraindividually stable and reflect the pathophysiological mechanism of lesion development (Lucchinetti et al. 2000; Metz et al. 2014). Pattern I and II share similar histopathological features. In these patterns inflammatory mechanisms seems to play the main role in lesion development. These patterns are only distinguishable from each other by the immunoglobulins and complement deposits along the myelin sheaths and within the macrophages observed in pattern II, suggesting an antibody/complement-mediated demyelination. However, specific pathogenic autoantibodies in MS patients could not yet be identified, although MOG-IgG antibodies may be pathogenic in a low percentage of adult pattern II patients (Konig et al. 2008; Di Pauli et al. 2015; Spadaro et al. 2015; Jarius et al. 2016).

Due to our findings we focused on the efficiency of apheresis therapies in MS patients stratified according to their pattern of early demyelination. Demographic data as well as clinical baseline characteristics stratified by immunopathological patterns are summarized in Table 3. Groups showed no statistically significant differences in most demographical and clinical parameters listed. However, disease course, time intervals between the start of the index attack and the apheresis therapy (PLEX/IA delay) and disease duration (time interval from first symptoms ever up to apheresis therapy) were different between the groups. To exclude possible influences of these parameters on primary and secondary outcome measures, analyses were corrected for these variables.

Table 3: Demographic and clinical characteristics of PLEX/IA cohort at the time of apheresis treatment stratified to the immunopathological pattern of MS lesions

	Pattern I	Pattern II	Pattern III	p-value
Sample size	n = 16	n = 40	n = 13	
Age: mean(sd)	35.3 (13.1)	38.4 (13.9)	32.7 (11.3)	0.376
Proportion of females (%)	10/16 (62.5%)	30/40 (75.0%)	6/13 (46.2%)	0.144

Disease course: Single clinical episode (%)	8/16 (50.0%)	11/40 (27.5%)	9/13 (69.2%)	0.022
Disease course: RR (%)	7/16 (43.8%)	26/40 (65.0%)	3/13 (23.1%)	0.024
Disease course: SP (%)	1/16 (6.2%)	3/40 (7.5%)	1/13 (7.7%)	1.000
Disease duration (years): median (min, max)	0.1 (0.0,17.0)	0.6 (0.0,18.0)	0.1 (0.0,16.0)	0.070
EDSS baseline: median (min, max)	0.0 (0.0,6.5)	2.0 (0.0,8.5)	0.0 (0.0,8.5)	0.198
EDSS at index attack: median (min, max)	7.5 (3.5,9.5)	5.0 (2.0,9.5)	6.0 (3.0,9.0)	0.120
PLEX/IA delay (days): mean(sd)	16.3 (13.6)	26.5 (22.8)	33.2 (16.1)	0.074
Therapy with HDCS before PLEX/IA (%)	13/16 (81.2%)	37/40 (92.5%)	13/13 (100.0%)	0.192
Therapy with DMD within 3 months before PLEX/IA (%)	5/15 (33.3%)	11/39 (28.2%)	1/13 (7.7%)	0.250

Abbreviations: RR: relapsing remitting; SP: secondary progressive; EDSS: expanded disability status scale; PLEX: plasma exchange; IA: immunoadsorption; HDCS: high dose of corticosteroids; DMD: disease-modifying drugs, sd: standard deviation, min: minimum, max: maximum. p-values < 10% are printed in bold. Reproduced with permission from JAMA Neurology 2018, 75(4): 428-435. Copyright© (2018) American Medical Association. All rights reserved.

The same three main outcome parameters (functional improvement, MRI and EDSS responses) were applied in this analysis. The highest response rate with 55% was found in pattern II patients (22/40 patients), fitting partially to the findings from Keegan et al. 2005 with a response rate of 100% in pattern II patients. In addition, we could show that every third patient with pattern I pathology (5/16 patients; pattern I vs pattern III p=0.03) also responded to the PLEX/IA therapy. Patients with pattern III (0/13; p<0.001) lesions did not show any treatment response. Approximately the same picture was observed by analyzing MRI improvement as an outcome parameter. Pattern II patients showed more often a lesion regression (56%; n=14/25) compared to pattern III patients (11%; n=1/9; p=0.03). In pattern I patients, lesion improvement was observed in 25% of patients (n=3/12). EDSS response again was highest in pattern II (40%; 15/38) followed by pattern I (25%; 4/16) patients. None of the patients with a pattern III showed an EDSS

improvement (0/13, Figure 5). These differences remained statistically significant after adjustment for the covariables disease duration, PLEX/IA delay, affection of the brainstem or cognitive system and therapy with immunoadsorption in a logistic regression model.

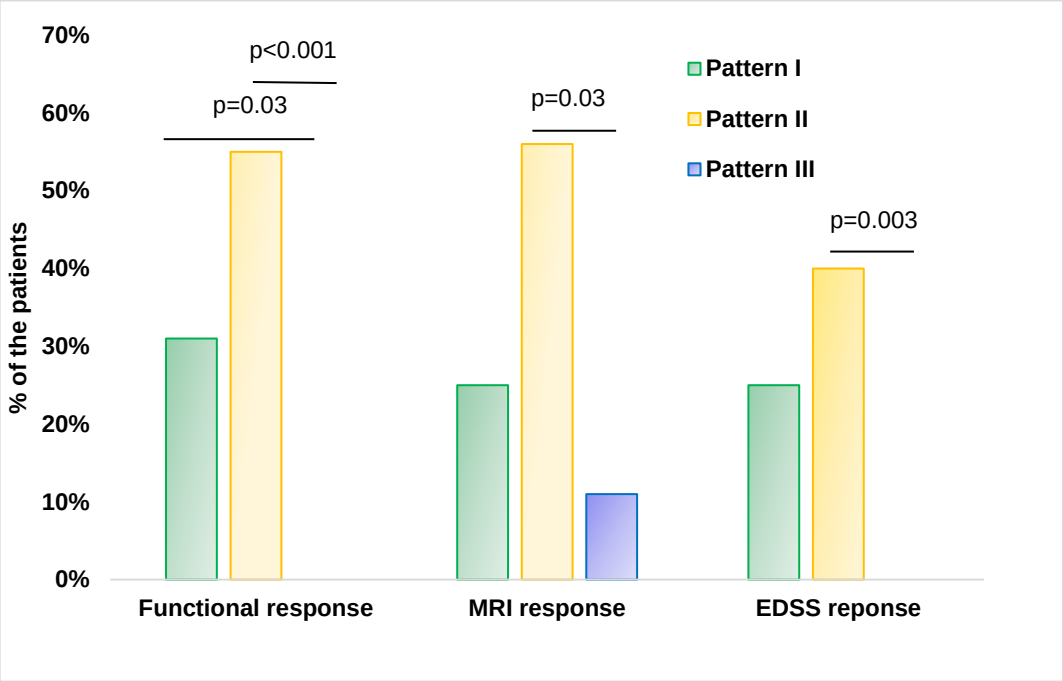

Figure 5: Functional, MRI and EDSS responses to apheresis therapies stratified according to immunpathological patterns of MS lesions.

The percentage of patients with functional (moderate or marked functional improvement), MRI (lesions that shrunk and/or showed less contrast enhancement) and EDSS (EDSS improvement ≥ 0.5 in patients with EDSS score ≥ 6.0 and an EDSS improvement ≥ 1.0 in patients with EDSS score ≥ 5.5) response is shown.

Nonspecific removal of antibodies and circulating immune complexes is suggested as a mechanism of action of apheresis therapies in pattern II patients. Apheresis therapies have been shown before to be beneficial in CNS antibody-mediated diseases such NMO and NMDA (N-methyl-D-aspartate) receptor encephalitis. Apheresis therapies reduce serum antibodies by 85% compared to pre-apheresis levels (Kim et al. 2013; Kleiter et al. 2016).

In pattern I lesions, proinflammatory mediators such as cytokines and chemokines produced by activated microglia/macrophages and T cells were suggested to cause myelin damage (Popescu et al. 2013). Elimination of cytokines, soluble cytokine receptors, adhesion molecules or complement factors from plasma may thus be beneficial in pattern I patients, but data on the removal of these substances with PLEX are controversial (Reeves and Winters 2014). Cytokine levels were not lowered after PLEX in septic patients (Hamishehkar et al. 2013). In contrast, a reduction in interleukin 8 (IL8) and tumor necrosis factor α (TNF-α) cytokine levels was observed after PLEX therapy for thrombotic thrombocytopenic purpura (TTP), but returned to pre-apheresis levels one day later

(Shariatmadar et al. 2005). Levels of soluble intracellular adhesion molecular 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) decreased after PLEX for myasthenia gravis (Tesar et al. 2000). Fibrinogen and C3 were reduced in plasma after PLEX for MS relapses (Weiner et al. 1989). Thus, elimination of factors other than antibodies may be relevant for the treatment effects of apheresis therapies observed in about one third of patients with pattern I pathology.

In addition to the removal of pathological agents, changes in immune cell numbers, composition and activation after apheresis treatment can also be observed. In patients with Guillain-Barré syndrome, B cell numbers decreased and T cells, particularly CD4+ T cells, increased after apheresis treatment, resulting in a normalization of cell subsets (Yoshi and Shinohara 2000). Suppressor functions of T helper cells increased after PLEX/IA in patients with chronic inflammatory demyelinating polyneuropathy (CIDP) to a level of healthy controls (De Luca et al. 1999). A shift in the balance of Th1/Th2 T cells was also described after PLEX treatment (Soltesz et al. 2002). Changes may occur either due to alterations in concentrations of soluble plasma factors, or due to the apheresis procedure itself: HLA (human leukocyte antigen)-I molecules adsorbed on the polymer membrane or absorber column may modulate the immune response of T lymphocytes and neutrophils during their bypass, resulting in their activation (Ghio et al. 2014). Although studies have not been performed in MS patients, immune cell alterations may also be relevant for the reduction of inflammatory activity in MS after apheresis treatments.

In contrast, the histopathology of pattern III lesions resembles white matter stroke, and the mitochondrial changes described in these lesions suggest a hypoxia-like tissue injury rather than an inflammation-driven pathogenesis (Mahad et al. 2008). This might explain the non-response to PLEX/IA treatment.

Fourteen patients received more than one PLEX/IA session. With the use of generalized estimation equations, longitudinal measurements of therapy responses to consecutive PLEX/IA sessions within one patient were not positively correlated (rho = -0.269). This means that prior therapy response in pattern I and II patients did not predict therapy response in later sessions. Patients with pattern III did not respond to either the first or the following PLEX/IA session.

3.5 Conclusions

In conclusion, this study shows that histopathological patterns I and II, involvement of the cognitive system with the clinical relapse, as well as application of IA could help to predict a therapy success with apheresis therapies in MS patients with steroid-resistant relapses. In contrast, brainstem involvement and histological features of immunopathological pattern III were negative predictive factors. Differences in the response to PLEX/IA comparing the immunopathological patterns I-III of MS lesions elucidates the potential mechanism of action of apheresis therapies, and may at least in part explain differences in the apheresis

response among MS patients. Importantly, if an apheresis treatment was not successful for the first relapse, it still may be effective for the next relapse.

4 Summary

Plasma exchange and immunoadsorption are second-line apheresis therapies for steroid-unresponsive multiple sclerosis relapses with a variable response rate. The mechanism of action of these therapies is assumed to be the removal of disease-causing agents such as antibodies, immune complexes and cytokines. A retrospective analysis of different demographical, clinical and histological parameters, which potentially could predict responses to apheresis therapies, was performed in 69 patients with multiple sclerosis lesions classified into pathological patterns I-III. The primary therapy outcome parameter was a functionally relevant improvement of the relapse-related neurological deficit. Radiological and expanded disability status scale changes were secondary outcome parameters.

We found that immunopathological patterns I and II, as well as application of immunoadsorption and involvement of the cognitive function with the relapse were positive predictive factors for a functional therapy response. In contrast, immunopathological pattern III and brainstem involvement with the relapse were negative predictive factors. A functional therapy response was observed in 31% (5/16) of pattern I and 55% (22/40) of pattern II patients, whereas no improvement was found in pattern III patients (0/13, $p<0.001$ pattern II versus III). Radiological findings supported the primary outcome. Lesion improvements were found in 25%, 56% and 11% of patterns I, II and III, respectively. The expanded disability status scale response rates again showed highest success rates in pattern II patients (40%) and were 25% and 0% for patients with patterns I and III.

Our results show that the response to apheresis treatment could be predicted by immunopathological patterns as well as involvement of the cognitive and brainstem systems. Potentially, IA is more effective than PLEX, but this has to be clarified in further studies. Different pathological subtypes of early active multiple sclerosis lesions suggests different pathophysiological mechanism of lesion development and thus may explain the varying therapy responses. Pattern I and II lesions show sharp lesion edges and an infiltration with T-cells and macrophages. Additionally, an antibody and complement-mediated mechanism of demyelination is suggested in pattern II. These patients also showed the most success from the apheresis treatment. In contrast, in pattern III lesions a primary oligodendrocytic damage may play an important role in lesion pathogenesis; patients showing this pattern are not amenable to apheresis treatments.

5 Supplementary material

The doctoral thesis was written based on the following original publication**:**

Stork L, Ellenberger D, Beißbarth T, Friede T, Lucchinetti C, Brück W, Metz I (2018): Differences in the responses to apheresis therapy of patients with 3 histopathologically classified immunopathological patterns of multiple sclerosis. JAMA. Neurol 75, 428-435.

To read the article please follow the link:

doi:10.1001/jamaneurol.2017.4842

This article received an acknowledgement from the editor Robert J. Fox

Fox RJ (2018): Tissue markers for the acute multiple sclerosis treatment response – a step towards personalized medicine. JAMA Neurol 75, 406-407.

To read this editorial please follow the link:

doi:10.1001/jamaneurol.2017.4850

Lidia Stork and Imke Metz also received an Apheresis Innovation Award from the German nephrological society for this study.

6 References

Aboul-Enein F, Rauschka H, Kornek B, Stadelmann C, Stefferl A, Bruck W, Lucchinetti C, Schmidbauer M, Jellinger K, Lassmann H (2003): Preferential loss of myelin-associated glycoprotein reflects hypoxia-like white matter damage in stroke and inflammatory brain diseases. J Neuropathol Exp Neurol 62, 25-33

Amato MP, Ponziani G (1999): Quantification of impairment in MS: discussion of the scales in use. Mult Scler 5, 216-219

Bevan C, Gelfand JM (2015): Therapeutic management of severe relapses in multiple sclerosis. Curr Treat Options Neurol 17, 345

Bruck W, Porada P, Poser S, Rieckmann P, Hanefeld F, Kretzschmar HA, Lassmann H (1995): Monocyte-macrophage differentiation in early multiple-sclerosis lesions. Ann Neurol 38, 788-796

Bruck W, Neubert K, Berger T, Weber JR (2001): Clinical, radiological, immunological and pathological findings in inflammatory CNS demyelination - possible markers for an antibody-mediated process. Mult Scler 7, 173-177

Cotton F, Weiner HL, Jolesz FA,Guttmann CR (2003): MRI contrast uptake in new lesions in relapsing-remitting MS followed at weekly intervals.Neurology 60, 640-646.

De Luca G, Lugaresi A, Iarlori C, Marzoli F, Di Iorio A, Gambi D, Uncini A (1999): Prednisone and plasma exchange improve suppressor cell function in chronic inflammatory demyelinating polyneuropathy. J Neuroimmunol 95, 190-194

Di Pauli F, Hoftberger R, Reindl M, Beer R, Rhomberg P, Schanda K, Sato D, Fujihara K, Lassmann H, Schmutzhard E, et al. (2015): Fulminant demyelinating encephalomyelitis: Insights from antibody studies and neuropathology. Neurol Neuroimmunol Neuroinflamm 2, e175

Ehler J, Koball S, Sauer M, Mitzner S, Hickstein H, Benecke R, Zettl UK (2015): Response to Therapeutic Plasma Exchange as a Rescue Treatment in Clinically Isolated Syndromes and Acute Worsening of Multiple Sclerosis: A Retrospective Analysis of 90 Patients. PLos One 10, e0134583

Faissner S, Nikolayczik J, Chan A, Hellwig K, Gold R, Yoon MS, Haghikia A (2016): Plasmapheresis and immunoadsorption in patients with steroid refractory multiple sclerosis relapses. J Neurol 263, 1092-1098

Filippi M, Rocca MA, Ciccarelli O, De Stefano N, Evangelou N, Kappos L, Rovira A, Sastre-Garriga J, Tintore M, Frederiksen JL, et al. (2016): MRI criteria for the diagnosis of multiple sclerosis: MAGNIMS consensus guidelines. Lancet Neurol 15, 292-303

Gajdos P, Chevret S, Toyka K (2002): Plasma exchange for myasthenia gravis. Cochrane Database Syst Rev 4 CD002275

Ghio M, Contini P, Ansaldi F, Ubezio G, Setti M, Risso M, Tripodi G (2014): A possible role of soluble HLA-I molecule in the immunomodulatory effects of therapeutic apheresis. Blood Transfus 12, 167-169

Guidline on clinical inverstigation of medical products for the treatment of Multiple Sclerosis. 2; hrsg. European Medical Agency, http://www.ema.europa.eu/ 2015; access on 12.05.2016

Hamishehkar H, Beigmohammadi MT, Abdollahi M, Mousavi S, Ziaie S, Sharifian RA, Davoudi S, Mojtahedzadeh M (2013): Pro-inflammatory cytokine profile of critically ill septic patients following therapeutic plasma exchange. Transfus Apher Sci 48, 75-78

Jarius S, Metz I, König FB, Ruprecht K (2016): Screening for MOG-IgG and other 27 anti-glial and anti-neuronal antibodies in "pattern II multiple sclerosis" amd brain biopsy findings in a MOG-IgG-positive cases. Mult Scler 22, 1541-1549

Keegan M, Pineda AA, McClelland RL, Darby CH, Rodriguez M, Weinshenker BG (2002): Plasma exchange for severe attacks of CNS demyelination: Predictors of response. Neurology 58, 143-146

Keegan M, König F, McClelland R, Brück W, Morales Y, Bitsch A, Panitch H, Lassmann H, Weinshenker B, Rodriguez M, et al. (2005): Relation between humoral pathological changes in multiple sclerosis and response to therapeutic plasma exchange. Lancet 366, 579-582

Kim SH, Kim W, Huh SY, Lee KY, Jung IJ, Kim HJ (2013): Clinical efficacy of plasmapheresis in patients with neuromyelitis optica spectrum disorder and effects on circulating anti-aquaporin-4 antibody levels. J Clin Neurol 9, 36-42

Kleiter I, Gahlen A, Borisow N, Fischer K, Wernecke KD, Wegner B, Hellwig K, Pache F, Ruprecht K, Havla J, et al. (2016): Neuromyelitis optica: Evaluation of 871 attacks and 1,153 treatment courses. Ann Neurol 79, 206-216

Koch-Henriksen N, Sorensen PS (2010): The changing demographic pattern of multiple sclerosis epidemiology. Lancet Neurol 9, 520-532

Konig FB, Wildemann B, Nessler S, Zhou D, Hemmer B, Metz I, Hartung HP, Kieseier BC, Bruck W (2008): Persistence of Immunopathological and Radiological Traits in Multiple Sclerosis. Arch Neurol 65, 1527-1532

Koziolek MJ, Tampe D, Bähr M, Dihazi H, Jung K, Fitzner D, Klingek R, Müller GA, Kitze B (2012): Immunoadsorption therapy in patients with multiple sclerosis with steroid-refractory optical neuritis. J Neuroinflammation 9, 2094-2099

Koziolek MJ, Kitze B, Muhlhausen J, Muller GA (2013): Immunoadsorption in steroid-refractory multiple sclerosis. Atheroscler Suppl 14, 175-178

Köhler W, Bucka C, Klingel R (2011): A randomized and controlled study comparing immunoadsorption and plasma exchange in myasthenic crisis. J Clin Apher 26, 347-355

Llufriu S, Castillo J, Blanco Y, Ramio-Torrenta L, Rio J, Valles M, Lozano M, Castella MD, Calabia J, Horga A, et al. (2009): Plasma exchange for acute attacks of CNS demyelination Predictors of improvement at 6 months. Neurology 73, 949-953

Lublin FD (2014): New Multiple Sclerosis Phenotypic Classification. Eur Neurol 72, 1-5

Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H (2000): Heterogeneity of multiple sclerosis lesions: Implications for the pathogenesis of demyelination. Ann Neurol 47, 707-717

Magana SM, Keegan BM, Weinshenker BG, Erickson BJ, Pittock SJ, Lennon VA, Rodriguez M, Thomsen K, Weigand S, Mandrekar J, et al. (2011): Beneficial plasma exchange response in central nervous system inflammatory demyelination. Arch Neurol 68, 870-878

Mahad D, Ziabreva I, Lassmann H, Turnbull D (2008): Mitochondrial defects in acute multiple sclerosis lesions. Brain 131, 1722-1735

McLeod BC (2010): Therapeutic apheresis: history, clinical application, and lingering uncertainties Transfusion 50, 1413-1426

Meca-Lallana JE, Hernandez-Clares R, Leon-Hernandez A, Genoves Aleixandre A, Cacho Perez M, Martin-Fernandez JJ (2013): Plasma exchange for steroid-refractory relapses in multiple sclerosis: an observational, MRI pilot study. Clin Ther 35, 474-485

Metz I, Weigand SD, Popescu BFG, Frischer JM, Parisi JE, Guo Y, Lassmann H, Bruck W, Lucchinetti CF (2014): Pathologic Heterogeneity Persists in Early Active Multiple Sclerosis Lesions. Ann Neurol 75, 728-738

Meyer-Moock S, Feng YS, Maeurer M, Dippel FW, Kohlmann T (2014): Systematic literature review and validity evaluation of the Expanded Disability Status Scale (EDSS) and the Multiple Sclerosis Functional Composite (MSFC) in patients with multiple sclerosis. BMC Neurol 14, 58

Moldenhauer A, Haas J, Wascher C, Derfuss T, Hoffmann KT, Kiesewetter H, Salama A (2005): Immunoadsorption patients with multiple sclerosis: an open-label pilot study. Eur J Clin Invest 35, 523-530

Niedziela N, Adamczyk-Sowa M, Pierzchala K (2014): Epidemiology and clinical record of multiple sclerosis in selected countries: a systematic review. Int J Neurosci 124, 322-330

Okafor C, Ward DM, Mokrzycki MH, Weinstein R, Clark P, Balogun RA (2010): Introduction and overview of therapeutic apheresis. J Clin Apher 25, 240-249

Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, Fujihara K, Havrdova E, Hutchinson M, Kappos L, et al. (2011): Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 69, 292-302

Popescu BF, Pirko I, Lucchinetti C (2013): Pathology of Multiple Sclerosis: Where do we stand? Continuum 4, 901-921

Reeves HM, Winters JL (2014): The mechanisms of action of plasma exchange. Br J Haematol 164, 342-351

Schimrigk S, Faiss J, Köhler W, Günther A, Harms L, Kraft A, Ehrlich S, Eberl A, Fassbender C, Klingel R et al. (2016): Escalation therapy of steroid refractory multiple sclerosis relapse with trypophan immunoadsorption - observation multicenter study with 147 patients. Eur neurol 75, 300-306

Schwartz J, Winters JL, Padmanabhan A, Balogun RA, Delaney M, Linenberger ML, Szczepiorkowski ZM, Williams ME, Wu Y, Shaz BH (2013): Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the Writing Committee of the American Society for Apheresis: the sixth special issue. J Clin Apher 28, 145-284

Shariatmadar S, Nassiri M, Vincek V (2005): Effect of plasma exchange on cytokines measured by multianalyte bead array in thrombotic thrombocytopenic purpura. Am J Hematol 79, 83-88

Shaygannejad V, Rezaie N, Paknahad Z, Ashtari F, Maghzi H (2016): The environmental risk factors in multiple sclerosis susceptibility: A case-control study. Adv Biomed Res 5, 98

Soltesz P, Aleksza M, Antal-Szalmas P, Lakos G, Szegedi G, Kiss E (2002): Plasmapheresis modulates TH1/TH2 imbalance in patients with systemic lupus erythematosus according to measurement of intracytoplasmic cytokines. Autoimmunity 35, 51-56

Sospedra M, Martin R (2005): Immunology of multiple sclerosis. Annu Rev Immunol 23, 683-747

Spadaro M, Gerdes LA, Mayer MC, Ertl-Wagner B, Laurent S, Krumbholz M, Breithaupt C, Hogen T, Straube A, Giese A, et al. (2015): Histopathology and clinical course of MOG-antibody-associated encephalomyelitis. Ann Clin Transl Neurol 2, 295-301

Stangel M, Fredrikson S, Meinl E, Petzold A, Stuve O, Tumani H (2013): The utility of cerebrospinal fluid analysis in patients with multiple sclerosis. Nat Rev Neurol 9, 267-276

Szczeklik W, Wawrzycka K, Wludarczyk A, Sega A, Nowak I, Seczynska B, Fajifer I, Zajac K, Krolikowski W, Kozka M (2013): Complications in patients treated with plasmapheresis in the intensive care unit. Anaesthesiol Intensive Ther 45, 7-13

Tesar V, Jelinkova E, Jirsa M, Bakosova M, Pitha P, Chabova V (2000): Soluble adhesion molecules and cytokines in patients with myasthenia gravis treated by plasma exchange. Blood Purif 18, 115-120

Tillema JM, Pirko I (2013): Neuroradiological evaluation of demyelinating disease. Ther Adv Neurol Disord 6, 249-269

Tintore M, Rovira A, Arrambide G, Mitjana R, Rio J, Auger C, Nos C, Edo MC, Castillo J, Horga A et al., (2010): Brainstem lesions in clinically isolated syndromes. Neurology 75, 1933-1938

Trojano M, Avolio C, Manzari C, Calo A, Derobertis F, Serio G, Livrea P (1995): Multivariate-analysis of predictive factors of multiple-sclerosis course with a valid method to assess clinical event. J Neurol Neurosurg Psychiatry 58, 300-306

Weiner HL, Dau PC, Khatri BO, Petajan JH, Birnbaum G, McQuillen MP, Fosburg MT, Feldstein M, Orav EJ (1989): Double-blind-study of true vs sham plasma-exchange in patients treated with immunosuppression for acute attacks of multiple-sclerosis. Neurology 39, 1143-1149

Weinshenker BG (1996): Epidemiology of multiple sclerosis. Neurol Clin 14, 291-308

Weinshenker BG, O'Brien PC, Petterson TM, Noseworthy JH, Lucchinetti CF, Dodick DW, Pineda AA, Stevens LN, Rodriguez M (1999): A randomized trial of plasma exchange in acute central nervous system inflammatory demyelinating disease. Ann Neurol 46, 878-886

Williams ME, Balogun RA (2014): Principles of separation: indications and therapeutic targets for plasma exchange. Clin J Am Soc Nephrol 9, 181-190

Yamada C, Teener JW, Davenport RD, Cooling L (2015): Maintenance plasma exchange treatment for muscle specific kinase antibody positive myasthenia gravis patients. J Clin Apher 30, 314-319

Yoshi F, Shinohara Y (2000): Lymphocyte subset proportions in Guillain-Barre syndrome patients treated with plasmapheresis. Eur Neurol 44, 162-167

Acknowledgements

I would like to express my sincere gratitude to my supervisor, **Prof. Dr. med. Imke Metz**, for guiding and supporting my doctoral dissertation, for her patience, motivation and immense knowledge. Her guidance has helped me during the entire period of research and writing of publications as well as this thesis work. She is an example of excellence as a researcher and mentor, and she always finds time for discussion and invaluable feedback.

Besides my advisor, I would also like to thank my co-supervisor, **Prof. Dr.med Katharina Hein**, and the director of our department, **Prof. Dr. med. Wolfgang Brück**, for their encouragement, insightful comments and tough questions.

My sincere thanks also goes to **Sven Müller** for his help with patient documentation, his friendly collegial support - as well for his useful advices. I would also like to thank **Mareike Gloth** for her technical assistance and the **members of our work group** for the friendly collegial atmosphere.

I would like to thank my beloved family and especially **my husband, Vadim Stork**, who makes it possible for me to pursue my research career by supporting and motivating me, and together with our children makes my life happy every day.

Finally, I would like to thank and to dedicate this thesis to my mother, **Dr. Irina Sviderskaya**. You are not only the person who gave a birth to me and who supports me spiritually through my life, but it was also you who originally generated my love for science. You have supported me during my first steps in research and you always give me valuable advice. I still take your lessons with me, every day.