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Primary progressive multiple sclerosis (PPMS) – cerebrospinal Fluid (CSF) profile

Dissertation to obtain the doctoral degree in medicine from the medical faculty of Ulm

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Abbreviations:

**BBB**: Blood Brain Barrier  
**BCB**: Blood-CSF-Barrier  
**CIS**: Clinically Isolated Syndrome  
**CNS**: Central Nervous System  
**CSF**: Cerebrospinal Fluid  
**DMT**: Disease Modifying Therapy  
**EDSS**: Extended Disability Score Scale  
**EDSS_{FU}**: EDSS to the time of the last follow-up  
**EDSS_{LP}**: EDSS to the time of Lumbar Puncture  
**EMA**: European Medicines Agency  
**FDA**: Federal Drug Agency  
**IEF**: Isoelectrical Focusing  
**Ig**: Immunoglobulin  
**IgX_{loc}**: Local Synthesis of Immunoglobulin (X) in CSF  
**LP**: Lumbar Puncture  
**LPD**: Lumbar puncture at the year of diagnosis  
**MAX**: Maximum  
**MRZ-Reaction**: Measles, Mumps and Rubella – Reaction  
**MS**: Multiple Sclerosis  
**OCB**: Oligoclonal Bands  
**PEG-INF**: pegylated-interferon  
**PMS**: Progressive Multiple Sclerosis  
**PPMS**: Primary Progressive Multiple Sclerosis  
**PPMS_T**: PPMS patients received Treatment  
**PPMS_{TN}**: Treatment-naïve PPMS patients  
**Q_{ALB}**: Albumin-Quotient  
**RRMS**: Relapsing Remitting Multiple Sclerosis  
**SPMS**: Secondary Progressive Multiple Sclerosis
1. Introduction:

Multiple sclerosis is the most common cause of neurological disability in young adult worldwide. The prevalence in Europe is 83 in 100,000 and incidence of 4.3 cases per 100,000 (Pugliatti et al. 2006). The disease was first described by Charcot (1825-1893) under the name sclerose en plaques (Compston 1988). Over the following years, many pathological studies described the inflammatory induced demyelinated plaques in the central nervous system as the hallmark of the disease (Noseworthy et al. 2000). Three main distinct clinical subtypes could be identified; relapsing remitting multiple sclerosis, secondary progressive multiple sclerosis, primary progressive multiple sclerosis and progressive relapsing multiple sclerosis (Lublin et al. 1996). Over the last years, the therapeutic options for the RRMS increased exponentially beginning with the approval of interferons injections for RRMS and ending with the approval of the PEG-INF in 2015 by the EMA and by the FDA (English et al. 2015). On the other hand, the available therapies for the progressive forms of multiple sclerosis (PMS) are are very limited. For SPMS, only the interferon B and Mitoxantrone showed efficacy in slowing the disease progression in SPMS (Martinelli Boneschi et al. 2005). Except very recently, all Disease modifying therapies (DMS) including corticosteroids, Interferons, Copaxone, Rituximab, Mitoxantrone and Fingolimod failed to slow the course

Development of the CSF examination in multiple sclerosis:

After performing the first lumbar puncture by Quincke, the CSF examination developed exponentially, and with the years it took its position in the diagnose and understanding the immune response in the CNS and the underlying pathology of MS (Uwe K Zettl et al. 2006). For a proper interpretation of the CSF results some basic concepts are essential (Reiber 2001, Reiber et al. 2001, Reiber et al. 2001, Reiber et al. 2011):

1. The Blood-CSF-Barrier (BCB): a term is compromising all the functional and anatomical processes influencing the final concentration of proteins in the CSF. This includes the blood-brain barrier in the capillary walls (BBB), protein secretion/ diffusion along the CSF flow path and more importantly the CSF flow rate. BCB dysfunction is a more accurate term than the BBB dysfunction and is more complex than the simple ‘leakage’ concept.

2. CSF proteins can be divided into three broad groups: pure blood-driven proteins like Albumin, proteins originating from Blood and within the central nervous system (CNS) like the immunoglobulins, proteins originating primarily from the CNS as well as from the neurones (S 100) or the leptomeninges (Beta-trace Protein, Cysteine C). Albumin is
suitable to assess the dysfunction of the BCB by dividing the CSF concentration over the serum concentration ($Q_{alb}$).

3. To determine the intrathecal fraction/production of a specific protein the degree of BCB dysfunction must be taken into account by referring the results to the $Q_{alb}$. However, the relation is non-linear, and the hyperbolic discrimination lines are considered a more accurate method to quantify the intrathecal synthesis.

4. The immune response of the CSF: the immunoglobulins in the CNS originate from blood and can be synthesised intrathecally by perivascular B lymphocytes. The intrathecal synthesis is polyspecific, i.e. directed against many antigens. It lasts for many years after the initial infection and lacks the well-known class-switch (i.e. from IgM to IgG).

The analysis protocol of CSF analysis as recommended by Reiber (Reiber et al. 2001) includes the following basic parameters: gross characteristic of CSF, total and differential cell count, total protein in CSF, Albumin, IgG, IgM, IgA in CSF and serum, IgG oligoclonal bands (OCB). The lactate and specific antibody indices (IgG class) can be measured when some diseases are suspected. Andersson et. al. (Andersson et al. 1994) divided the relevant CSF parameters in MS into two categories; one essential parameter which is the detection of
OCB and three complimentary parameters (cell count, $Q_{\text{alb}}$ and increased IgG-Index or IgG local synthesis in mg/l).

The cell count is optimally to be counted within 30-60 min of the lumbar puncture. The upper reference limit is four cell/µl. Most of MS patients show a normal cell count but results up to 50 cell/µl can still be seen in patients with MS (Reiber et al. 1998, Freedman et al. 2005).

The total CFS protein is not a very reliable method to measure the BCB as some of the CSF proteins originate from the Brain or the leptomeningeal tissues in comparison to Albumin, which is exclusively synthesised in the liver (Andersson et al. 1994, Reiber et al. 2001). Thus, the $Q_{\text{alb}}$ (Albumin in CSF/Albumin in serum) is widely accepted as the most reliable parameter for a ‘leaked’ Blood-CSF Barrier (BCB) (Felgenhauer et al. 1976). In MS patients usually, reveal normal or slightly elevated results (Eickhoff et al. 1977). The combination of high cell count and elevated $Q_{\text{alb}}$ usually indicates other inflammatory diseases but not MS (Reiber et al. 2001).

The intrathecal immunoglobulins synthesis is a hallmark in MS (Bonnan 2015). Detecting the OCB using the isoelectrical focusing (IEF) is considered the most sensitive test to quantitatively detect the intrathecal IgG synthesis in multiple sclerosis (Freedman et al. 2005) The sensitivity reached up to 100% in one study (Kostulas et al. 1987). However, they are not highly specific for multiple
sclerosis (Beer et al. 1995). The resulted patterns are described in Table 1 (Andersson et al. 1994, Freedman et al. 2005).

<table>
<thead>
<tr>
<th>Pattern 1</th>
<th>no bands in CSF or serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pattern 2</td>
<td>bands detected in CSF only</td>
</tr>
<tr>
<td>Pattern 3</td>
<td>bands in CSF plus identical bands in CSF and serum</td>
</tr>
<tr>
<td>Pattern 4</td>
<td>identical bands in CSF and serum</td>
</tr>
<tr>
<td>Pattern 5</td>
<td>monoclonal bands in CSF and serum</td>
</tr>
</tbody>
</table>

Patients with OCB have reached a higher EDSS score in some studies or were associated with poor recovery from relapses. Nevertheless, other studies revealed no prognostic values of the OCB (Dobson et al. 2013). The quantitative measuring of the intrathecal IgX-index, the link index (LI) (IgX index = QIgX/Qalb) correlates the intrathecal IgX amount to the degree of BCB dysfunction (Link et al. 1977). Reiber defined the intrathecal synthesis as the amount of IgX above the Qlim (Reiber et al. 1987, Reiber et al. 2001). The Qlim represents the uppermost limit of the normal values and can be determined using the following equation: $Q_{lim} = \frac{a}{b} \sqrt{Q_{ALB}}^2 + b^2 - c$ where $a/b$, $b^2$ and $c$ values for every immunoglobulin differs (Reiber et al. 2001). The intrathecal fraction (IFIgX) in % = $(1-Q_{lim}/Q_{IgX}) \times 100$ and the absolute amount in mg/l = $(Q_{IgX} - Q_{lim}) \times IgX_{serum}$. IF > 10% and positive values in mg/l are considered the most unbiased quantitative measurements of the intrathecal IgX synthesis (Bonnan 2015). The IgG-index or $IgG_{loc}$ is elevated in 80-90% of MS patients.
(Andersson et al. 1994). The quantitative intrathecal synthesis was associated with faster disease progression in some studies (Stendahl-Brodin et al. 1980, Izquierdo et al. 2002), albeit not all (Lourenco et al. 2013).

Other optional parameters in the CSF of MS patients include the MRZ reaction (Measles, Mumps and Zoster) as a marker for the polyspecific immune response (see above) (Reiber et al. 1998). The frequency of intrathecal immune reaction against measles, rubella and varicella zoster was much higher any other neurological disease (Felgenhauer et al. 1992). To measure the fraction of antibodies (AB) against a specific pathogen from the total intrathecal immunoglobulin synthesis, the specific antibody index (AI) one can divide the $Q_{\text{spec}}$ over $Q_{\text{IgG}}$ or over $Q_{\text{lim}}$ if $Q_{\text{lim}} < Q_{\text{IgG}}$ (Reiber et al. 1991), where $Q_{\text{spec}} = \frac{AB_{\text{CSF}}}{AB_{\text{serum}}}$. The result is considered pathological if the AI > 1.4.

In MS the frequency of AI against M/R/Z (and/or) is about 89%. However, if a complete positive MRZ reaction ($\geq$ two positive AI) can be found in only 60-70% of MS patients (Bednarova et al. 2005, Brettschneider et al. 2009, Rosche et al. 2012). The MRZ reaction is thought to be a product of the tertiary lymphoid organs in the meninges (Bonnan 2014). The MRZ reaction is considered a very specific parameter in MS (Reiber et al. 1998). MRZ reaction can predict the conversion of CIS patients to MS (Brettschneider et al. 2009).

The relevance of CSF-lactate level in MS was ignored till recently. The CSF-lactate in mmol/l is elevated as a result of the anaerobic metabolism occurring
in bacterial infections and after convulsions (Leen et al. 2012). In MS the CSF-lactate usually fall within the normal range (Lutz et al. 2007, Amorini et al. 2014). However, a recent study reported a positive correlation between the CSF-lactate and the disease progression rate in 118 RRMS patients (Albanese et al. 2016). Moreover, a previous study reported a positive correlation between serum lactate and the disease severity in all clinical subtypes of multiple sclerosis (Amorini et al. 2014). Furthermore, a positive correlation between the CSF-lactate and number of inflammatory MS plaques was reported in another study with 33 CIS patients (Lutz et al. 2007). This correlation could be explained with the well described mitochondrial dysfunction in MS, especially in the progressive forms (Lassmann 2014, Witte et al. 2014).

CSF profile in PPMS: Many studies and review regarding the CSF parameters in MS were published over the last 50 years. Some of them included PPMS patients. Table 2 provides an overview of the published results.
Table 2: Studies reported cerebrospinal fluid (CSF) parameters from primary progressive multiple sclerosis (PPMS) patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of PPMS patients</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROMiSe Trial Study Group (Wolinsky et al. 2007)</td>
<td>943</td>
<td>78% of the patients have positive OCB and/or elevated IgG-index and/or increased local synthesis. Only the results of OCB have been reported.</td>
</tr>
<tr>
<td>McLean et al. (McLean et al. 1990)</td>
<td>31 (probable and possible PPMS)</td>
<td>OCB in 88% of the patients with more common pattern 2 than 3</td>
</tr>
<tr>
<td>Villar et al. (Villar et al. 2005)</td>
<td>39</td>
<td>64 % of the patients have pattern 3 OCB</td>
</tr>
<tr>
<td>Izquierdo et al. (Izquierdo et al. 2002)</td>
<td>23</td>
<td>Mean IgG-Index is 0.81 +/- 0.5. Higher IgG-Index is associated with faster disease progression</td>
</tr>
<tr>
<td>Reboul et al. (Reboul et al. 1995)</td>
<td>29 (chronic progressive MS)</td>
<td>Elevated IgM-index in 32.2%</td>
</tr>
<tr>
<td>Hottenrott, 2016 (Hottenrott et al. 2017)</td>
<td>103</td>
<td>Positive MRZ reaction in slightly more than the half of the patients</td>
</tr>
</tbody>
</table>

The aim and Hypothesis of our Study:
In contrast to RRMS, the CSF examination remains a part of the diagnostic criteria of PPMS. Thus a profound characterization of the CSF profile in PPMS is of diagnostic importance. The above mentioned previous studies share some drawbacks: relatively small number of patients included, assessing only
one aspect of the CSF profile (e.g., immunoglobulin synthesis) and the inclusion of some SPMS patients. Moreover, all those studies included patients who were diagnosed according to out-of-date diagnostic criteria. Analysis of the CSF profile in a large multicentric, pure PPMS cohort is our aim. The hypothesis our study postulates that the analysis of the baseline CSF profile of a well characterised, pure PPMS multicentric cohort will correlate with disease progression. This may reveal new aspects which might deviate from the previously reported results and can consequently be integrated into the upcoming versions of diagnostic guidelines.
2. Methodology:

Data collection:
CSF data was collected from four university hospitals Germany (Ulm, Frankfurt, Rostock and Freiburg). We included PPMS patients treated in-house or as outpatients between 2010 and 2015. The patients of our cohorts were either seen as inpatients due to admission for diagnostic workup ultimately leading to PPMS diagnosis, for application of medical therapy, or for inpatient rehabilitation or they visited the outpatient clinic for confirmation of the diagnosis or for follow up. Diagnosis of PPMS was established according to the 2010 revised McDonald criteria (Polman et al. 2011) after careful exclusion of relevant differential diagnoses. Lumbar puncture (LP) was performed for diagnostic purposes only and after written consent of all patients. CSF and serum samples were taken on the same day and stored according to consensus protocol for the standardisation of cerebrospinal fluid collection and biobanking (Teunissen et al. 2009). Hemolytic CSF specimens were excluded. Records of all available patients matching these criteria were retrospectively reviewed regarding age at onset, initial neurological complaints, age at first diagnosis, time between onset and diagnosis, expanded disability status scale (EDSS) at the time of lumbar puncture (EDSS$_{LP}$) and EDSS at the last documented follow-up (EDSS$_{FU}$) and therapies. Age at clinical onset and initial complaints were assessed according to the first
documented neurological symptoms, attributable to the disease and were obtained from the available medical records. We divided the initial complains in four main categories: motor, sensory, cerebellar and other symptoms. The category “others” comprised brain stem syndromes, visual disturbances, cognitive symptoms, convulsions and urinary manifestation. Clinical severity of MS disease was assessed using the EDSS (Kurtzke 1983). We calculated the progression rate by dividing \( \text{EDSS}_{FU} \) over the period between clinical onset and date of the \( \text{EDSS}_{FU} \). The \( \text{EDSS}_{LP} \) was either documented or has been retrospectively defined by a certified EDSS-rater.

The CSF analysis included basic parameters like total cell count, CSF-serum quotient for albumin (\( Q_{\text{ALB}} \)), Immunoglobulin G, M and A (IgG, IgM, IgA), lactate concentration, oligoclonal bands (OCB) pattern (McLean et al. 1990) and measles, rubella and zoster (MRZ)-reaction (Reiber et al. 1998). In cases of repeated lumbar puncture of the same patient, only the results of the one used to establish the diagnosis were analysed. \( Q_{\text{ALB}} \) was used as an indicator for the blood-CSF barrier (BCB) (Reiber 1994) and assessed according to the age-related reference range (age+15/4) (Reiber et al. 1998, Reiber et al. 2001). IgX-index was calculated using the Reiber formula (IgX CSF /IgX Serum: Albumin CSF /Albumin Serum) (Link et al. 1977). IgG-index values > 0.7 (Zettl UK 2003) and IgM-index values > 0.061(Forsberg et al. 1984) and IgA-index values >0.34 (Lolli et al. 1989) were considered as elevated. The quantitative
intrathecal IgG-synthesis (IgGloc) was calculated according to the following formula \( IgX_{loc} = (Q_{IgX} - Q_{lim}) \). IgXserum. The \( (Q_{lim} = a/b \sqrt{Q_{ALB}^2 + b^2} - c) \) where \( a/b, \ b^2 \) and \( c \) values for each immunoglobulin were determined according to the Reiber-formula (Reiber et al. 1987, Reiber et al. 1998, Reiber et al. 2011). Negative values are reported as zero (Reiber et al. 2001). IgG-oligoclonal bands (OCB) were classified corresponding to the above mentioned 5 patterns (Andersson et al. 1994, Freedman et al. 2005).

We considered MRZ-reaction as positive when the reported antibody index (AI) against at least two of the included indices was > 1,4 (Brettschneider et al. 2009, Hottenrott et al. 2015).

We classified our PPMS patients in two different categories; PPMS\(_T\): patients who received medical therapy over the course of the disease and PPMS\(_{TN}\): treatment naïve patients. Furthermore, according to the duration between the first complaint and LP, patients were further assigned to the following groups: LP\(_{\leq 5}\) LP was performed within the first 5 years of the disease, within 5-10 years (LP\(_{5-10}\)) and more than 10 years (LP\(_{>10}\)). LP\(_D\): LP at the year of diagnosis, before any medical treatment has been started Table 3.
Table 3: The subgrouping of the included primary progressive multiple sclerosis (PPMS) patients:

We included 254 patients in our study and performed subgroup assignment and analysis according to multiple factors like the administration of medical treatment (treatment group PPMS\(_T\) and treatment-naïve patients PPMS\(_{TN}\)) and the period between initial manifestation and the lumbar puncture (LP) in years into LP \(\leq 5\), LP \(5-10\) and LP \(>10\). The LP\(_D\) subgroup included data from the LP from the same year of diagnosis.

<table>
<thead>
<tr>
<th>Time period between clinical onset and LP</th>
<th>Less than 5 years (LP (\leq 5))</th>
<th>Between 5 and 10 years (LP 5-10)</th>
<th>More than 10 years (LP &gt;10)</th>
<th>Total number of patients</th>
<th>Lumbar puncture at the year of diagnosis (LP(_D))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment naïve (PPMS(_{TN}))</td>
<td>53</td>
<td>17</td>
<td>17</td>
<td>87</td>
<td>82</td>
</tr>
<tr>
<td>With treatment (PPMS(_T))</td>
<td>107</td>
<td>17</td>
<td>27</td>
<td>151</td>
<td>125</td>
</tr>
<tr>
<td>Total number of patients</td>
<td>160</td>
<td>34</td>
<td>44</td>
<td>238 (missing data = 16)</td>
<td>207</td>
</tr>
</tbody>
</table>

The study was reviewed by the appropriate ethics committee in Ulm (approval number: 20/10) and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

**Statistics:**
All statistical tests were performed using IBM® SPSS® Statistics version 21 (Armonk, USA). We used Shapiro-Wilk test to examine the distribution of the date and Mann-Whitney U test to compare between the median of different
variables. Fisher’s exact test was used for qualitative variants and the spearman’s rho test to measure correlation. A p-value ≤ 0.05 was considered as statistically significant.
3. Results:

**Clinical characteristic of the Patients:**
The clinical features of the enrolled 254 PPMS patients are summarised in Table 4 and Figure 1.

<table>
<thead>
<tr>
<th>Clinical features of primary progressive multiple sclerosis (PPMS) patients (median, range)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>254</td>
</tr>
<tr>
<td>Age at first symptom</td>
<td>44 (15-72)</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>47 (22-77)</td>
</tr>
<tr>
<td>Time span between first symptom and diagnosis in years</td>
<td>3 (1-39)</td>
</tr>
<tr>
<td>Expanded disability status scale (EDSS) at the date of lumbar puncture (LP)</td>
<td>4.0 (1.0-8.5)</td>
</tr>
<tr>
<td>Follow up period in months</td>
<td>27 (1-197)</td>
</tr>
<tr>
<td>EDSS at follow up</td>
<td>6.0 (1.0-9.0)</td>
</tr>
</tbody>
</table>

*Figure 1: Distribution of the initial manifestations in a cohort of 251 primary progressive multiple sclerosis patients who visited university hospitals of Ulm, Freiburg, Rostock or Frankfurt between 2010 and 2014: Motor weakness was the most common initial symptom in about 71.4 % (175/245) of patients included in our multicentric cohort, followed by cerebellar manifestations in 14.7% (36/245) and sensory disturbances in 8.6 % (21/245). Other initial manifestations such as brain stem syndromes, visual disturbances, cognitive symptoms, convulsions or urinary disturbances were present in 6.3 % (13/245), source: (Abdelhak et al. 2017)*
PPMS\textsubscript{Motor} showed a worse EDSS\textsubscript{LP} compared to patients with PPMS\textsubscript{Cerebellar} or PPMS\textsubscript{Sensory} (4.0 vs 3.5 or 3.5, p < 0.001, n= 201). The EDSS progression rate did not differ significantly between these three subgroups with different initial symptoms (p> 0.5). Analysis of PPMS\textsubscript{TN} revealed similar results. The differences in EDSS\textsubscript{LP} between PPMS\textsubscript{Motor}, PPMS\textsubscript{Cerebellar} and PPMS\textsubscript{Sensory} were significant (4.5 vs 3.5 vs 4.0, p= 0.03). The EDSS progression rate did not statistically differ between the groups (0.80 vs 0.40 vs 0.32 respectively, p= 0.2).

Overall 154 patients received one or more of the following therapies over the course of the disease: 3-monthly pulse steroid therapy (n=71), Mitoxantrone (n= 29), Cyclosporine (n=1), Rituximab (n=6), Intravenous immunoglobulins (n=1), Dimethyl fumarate (n=1), Interferon beta-1a (n=2), Glatiramer acetate(n=1), Azathioprine (n=4), and more than one therapy (n=38). However, we did not include details of the therapies, like the duration, because it was not the focus of this study.

CSF profile in PPMS:

Cell count, total protein, albumin and CSF-lactate:

In a number of patients, some of the clinical and of CSF data were not obtainable from the records or could not be retrospectively evaluated. In our cohort, the median cell count was normal with 2 cells/µl (max: 101, n= 234).
Median CSF total protein was mildly elevated with 485 mg/l (max: 1775, n = 233). However, the CSF albumin content (median = 252mg/l, max: 1300, n = 176) was within the normal range. CSF-lactate was not elevated (median: 1.7, max: 5.6, n = 101). Age-matched Q\textsubscript{ALB} was elevated in 29.6 % (n=67/226) of our patients.

The LP\textsubscript{D} subgroup revealed similar results; the median cell count was 3 (max: 101, n= 152), median CSF protein = 485 mg/l (max: 1775, n= 153), median CSF albumin = 240 mg/l (max: 1300, n= 112), median lactate = 1.7 (max: 2.9, n= 63). The Q\textsubscript{ALB} was elevated in 32.7 % (n= 48/147).

The incidence of the elevated Q\textsubscript{ALB} decreased with the duration of the disease. Q\textsubscript{ALB} was elevated in 32.7 % (48/147) in the LP\textsubscript{≤5} group, in 25% LP\textsubscript{5-10} (8/32) and 22.7% of the LP \textsubscript{>10} (10/44). In PPMS\textsubscript{TN}, an elevated Q\textsubscript{ALB} was not significantly different in the LP \textsubscript{≤5}, LP\textsubscript{5-10} and LP \textsubscript{>10} subgroups (12.5 %; 36.2 % and 23.5%, p > 0.1).

**Intrathecal immunoglobulin synthesis:**

In 8.2 % (n=18/220) of the patients, no oligoclonal bands were found in CSF (pattern 1). Isolated IgG OCB in CSF (pattern 2) were the most common in our patients (78.6%, n=173/220). IgG OCB in CSF with additional identical bands in CSF and serum (pattern 3) were detected in 12.3% (n=27/220). We found two cases of identical IgG OCB in serum and CSF (pattern 4). The MRZ reaction was
positive in 56.8% (83/146) of the available results. MRZ reaction was positive in 6 of 9 patients with negative OCB (5 with pattern 1 and 1 with pattern 4 OCB).

The IgG index was elevated in 49.2 % (n=88/179) with a median IgG-index of 0.7 (max= 6.0). IgGloc was positive in 61.7 % with a median of 2.7 mg/l (max= 271.7 mg/l, n= 103/167).

Analysis of the LP0 subgroup revealed similar results. The IgG-index was elevated in 51.4 % of the patients (n=74/144) with a median of 0.8 (max= 6.0). IgGloc was positive in 63.1% and a median of 3.3 mg/l (max= 271.7 mg/l, n=89/141).

Comparing the incidence and amount of intrathecal IgG-production between LP≤5, LP5-10 and LP>10 revealed the following results Table 5.

<table>
<thead>
<tr>
<th>Total</th>
<th>LP≤5</th>
<th>LP5-10</th>
<th>LP&gt;10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Elevated in n/total (%)</td>
<td>Median (max)</td>
<td>Elevated n (%)</td>
</tr>
<tr>
<td>IgG-index</td>
<td>72/112 (64.3%)</td>
<td>0.8 (6.0)</td>
<td>14/26 (53.8%)</td>
</tr>
<tr>
<td>IgGloc</td>
<td>65/112 (58.0%)</td>
<td>3.7 (271.0)</td>
<td>14/26 (53.8%)</td>
</tr>
</tbody>
</table>
The median IgM index was 0.08 (max=1.0, n=176) which was elevated in 58.5% (103/176). The IgM_{loc} was positive in 21.0% (37/176) with a median IgM_{loc} of 0 mg/l (max= 4.5, n= 176). The median IgA index was 0.3 (max= 2.3, n= 171), while IgA index was elevated in 24.4% (43/176). IgA_{loc} was detectable in 17.6% of the patients (31/176), and the median IgA_{loc} was 0 mg/l (max= 72.7, n= 176).

The relation between CSF findings and clinical parameters:

The $Q_{ALB}$:

Analysing the whole group revealed no difference between median EDSS_{LP} (4.0, n= 198) and median progression rate (0.58, n= 137) in patients with normal and elevated $Q_{ALB}$. LP_{D} showed similar results with median EDSS_{LP} of 4.0 (n=129). In PPMS_{TN} the median progression rate was 0.73 and 0.54 in patients with normal and elevated $Q_{ALB}$ respectively (p= 0.6, n= 34).

CSF-lactate:

Levels of CSF-lactate showed consistently a positive correlation with the yearly progression rate in the entire cohort, LP_{D} and PPMS_{TN}. After excluding the one case with extremely high lactate levels (5.6 mmol/L), a statistically significant positive correlation between CSF-lactate and EDSS_{LP} was found only in LP_{D} but not in the entire Cohort or PPMS_{TN} Table 6 and Figure 2.
Table 6: Spearman correlation ($\rho$) between cerebrospinal fluid (CSF)-lactate and expanded disability score scale (EDSS) at the Lumbar Puncture (EDSS$_{LP}$) and the yearly progression rate in the entire cohort, Treatment-naive primary progressive multiple sclerosis (PPMS) patients (PPMS$_{TN}$) and at the LP at the year of diagnosis (LP$_{D}$)

<table>
<thead>
<tr>
<th></th>
<th>Entire cohort</th>
<th>PPMS$_{TN}$</th>
<th>LP$_{D}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation between CSF-lactate in (mmol/L) and EDSS$_{LP}$</td>
<td>$\rho$ = 0.2 (p = 0.07) (n=80)</td>
<td>$\rho$ = 0.2 (p = 0.3) (n=33)</td>
<td>$\rho$ = 0.3 (p = 0.03) (n=66)</td>
</tr>
<tr>
<td>Correlation between CSF-lactate in (mmol/L) and yearly progression rate</td>
<td>$\rho$ = 0.3 (p = 0.02) (n=76)</td>
<td>$\rho$ = 0.5 (p = 0.006) (n=32)</td>
<td>$\rho$ = 0.3 (p = 0.01) (n=62)</td>
</tr>
</tbody>
</table>
Spearman correlation between CSF-lactate and EDSS\(_{LP}\) in the entire cohort

Spearman correlation between CSF-lactate and the yearly progression rate in the entire cohort

Spearman correlation between CSF-lactate from the lumbar puncture from the year of diagnosis (LP\(_D\)) and EDSS\(_{LP}\)

Spearman correlation between CSF-lactate from the lumbar puncture from the year of diagnosis (LP\(_D\)) and yearly progression rate
In the entire cohort we found no difference between median EDSS_{LP} and the five OCB patterns (4.5 vs 4.0 vs 4.0, p= 0.8, n =194) but only a trend towards higher median progression rate in pattern 3 compared to 2 and 1 (0.80 vs 0.48 vs 0.47, p= 0.1, n= 126). In PPMS_{TN} the median EDSS_{LP} was 3.8 in patients with pattern 1 (n=4), pattern 2 was 4.0 (n= 114) and 4.3 in pattern 3 (n= 14). The difference was not statistically significant (p= 0.7). Also in the PPMS_{TN} we observed a trend towards higher yearly progression rate in pattern 3 OCB compared to 2 and 1 (0.80 vs 0.65 vs 0.29, p= 0.07, n= 34).
The EDSS_{LP} and the yearly progression rate did not differ significantly between patients with elevated and normal IgG/A/M index.

Using the Spearman correlation, we found no statistically significant correlation between the parameters of IgG production and the clinical parameter in the entire cohort and LP_{D}. Only in PPMS_{TN} we found a moderate positive correlation between quantitative markers of intrathecal IgG-synthesis and yearly progression rate Table 7.

<table>
<thead>
<tr>
<th>Parameter Correlation, p-value (number)</th>
<th>IgG-index</th>
<th>IgG_{loc}</th>
<th>Q_{ALB}</th>
<th>IgG-index (LP_{D})</th>
<th>IgG_{loc} (LP_{D})</th>
<th>Q_{ALB} (LP_{D})</th>
<th>IgG-index (PPMS_{TN})</th>
<th>IgG_{loc} (PPMS_{TN})</th>
<th>Q_{ALB} (PPMS_{TN})</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDSS_{LP}</td>
<td>-0.06, 0.5 (149)</td>
<td>-0.09, 0.3 (147)</td>
<td>-0.01, 0.9 (198)</td>
<td>-0.08, 0.4 (126)</td>
<td>-0.09, 0.3 (124)</td>
<td>-0.03, 0.8 (167)</td>
<td>0.02, 0.9 (49)</td>
<td>0.05, 0.7 (48)</td>
<td>-0.11, 0.4 (65)</td>
</tr>
<tr>
<td>EDSS_{FU}</td>
<td>-0.08, 0.4 (124)</td>
<td>-0.13, 0.2 (123)</td>
<td>0.05, 0.6 (141)</td>
<td>-0.01, 1.0 (32)</td>
<td>0.04, 0.8 (32)</td>
<td>-0.02, 0.9 (38)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yearly progression rate</td>
<td>0.02, 0.9 (120)</td>
<td>0.1, 0.3 (120)</td>
<td>-0.1, 0.3 (138)</td>
<td></td>
<td></td>
<td></td>
<td>0.4, 0.01 (35)</td>
<td>0.4, 0.02 (35)</td>
<td>-0.02, 0.9 (35)</td>
</tr>
</tbody>
</table>

In patients with intrathecal IgM/A synthesis, we found a moderate negative correlation between both IgM_{loc}, IgA_{loc}, and the yearly progression rate (ρ = -0.4, p = 0.03, n = 28, and ρ = -0.5, p = 0.01, n = 25, respectively).
4. Discussion:
Our study is, to the best of our knowledge, the largest hitherto reported CSF cohort with 254 PPMS patients. Motor impairment was the most frequent initial symptom followed by cerebellar disturbances and sensory manifestations, which is consistent with other published cohorts (Thompson et al. 1997, McDonnell et al. 1998, Cottrell et al. 1999, Miller et al. 2007, Rice et al. 2013). The higher EDSS\text{LP} in PPMS\text{Weakness} is probably due to the relatively large impact of motor symptoms on the overall EDSS (Meyer-Moock et al. 2014).

The basic CSF parameters such as cell count and albumin did not deviate from the normal values, which has previously been reported from other CSF cohorts in MS (Tourtellotte et al. 1978). The overall low incidence of elevated $Q_{\text{ALB}}$, i.e. blood-CSF barrier (BCB) dysfunction, is in accordance with the hypothesis of compartmentalization of the inflammation behind an intact blood-brain barrier (BBB) (Meinl et al. 2008). We observed a trend towards a further decrease of $Q_{\text{ALB}}$ over the disease duration possibly indicating either further compartmentalization or a decline in the severity of inflammation-induced dysfunction of the BCB. Indeed, this was reported in a histopathological study of postmortem brain samples of progressive MS patients (Frischer et al. 2009).
This questions the pathophysiological plausibility and therefore usefulness of the systemically applied therapies compared to the intrathecal modalities in late stages of the disease and could explain the limited success of intravenous rituximab (Hawker et al. 2009) and the negative results of other systemically applied therapies (Cazzato et al. 1995, Miller et al. 2004, Montalban 2004, Stuve et al. 2004, Wolinsky et al. 2007).

Until recently, the clinical significance of CSF lactate in MS was not known (Albanese et al. 2016). Thus, in more than the half of our patients, CSF-lactate levels had not been routinely measured. Nevertheless, we have reported a normal CSF-lactate median in patients with PPMS (Leen et al. 2012). Simone et al. (Simone et al. 1996) and Regenold et al. (Regenold et al. 2008) reported increased levels of lactate in MS patients, while Aasly et al. (Aasly et al. 1997) reported lower levels compared to healthy controls. However, all three studies did not include PPMS patients.

We have reported a weak to moderate positive correlation between CSF-lactate levels and the yearly progression rate in the entire cohort and all subgroups. A similar association was reported recently in a study with 118 RRMS patients (Albanese et al. 2016). A previous study also reported a positive correlation between serum lactate and the disease severity in all clinical subtypes of MS (Amorini et al. 2014). In other studies, CSF lactate correlated
with the cell count, inflammatory and gadolinium-enhancing MRI lesions in MS patients (Simone et al. 1996, Lutz et al. 2007). This might be explained through the mitochondrial dysfunction in progressive MS subtypes. Mitochondrial dysfunction with subsequent cellular hypoxia is especially relevant for the neurodegeneration of susceptible, chronically demyelinated axons that are commonly found in progressive MS subtypes (Trapp et al. 2009). However, this is rather speculative and prospective studies with a larger number of patients are essential to validate the prognostic value of testing CSF-lactate levels in PPMS.

The higher incidence of intrathecal IgG production indicated by OCBs compared to the elevated IgG-index and IgG_{loc} confirms the well-known higher sensitivity of isoelectric focusing (IEF) (Lunding et al. 2000). Our results were consistent with McLean et al.’s study, which reported OCB in 86–88% of samples from a cohort that included 31 progressive MS patients (McLean et al. 1990), but higher than those reported from the PROMiSe trial cohort (80%) (Wolinsky et al. 2007). OCBs pattern 2 was found the most frequently in our PPMS patients (79% of patients), matching the results of McLean et al.’s study (McLean et al. 1990). In contrast, Villar et al. observed a predominance (64%) of OCBs pattern 3, but the sample size was much smaller than that of our study (n = 39) (Villar et al. 2005). The role of systemic inflammation in the disease progression of PPMS is described elsewhere (Ukkonen et al. 2007).
(Romme Christensen et al. 2013). In our study, we did not find any differences between yearly progression rates in patients with OCBs patterns 2 and 3. Nevertheless, studies with a larger sample size are needed to confirm our results.

In our study, the frequency of elevated IgG-index values was lower than in the cohort described by Izquierdo et al. (Izquierdo et al. 2002). However, Izquierdo et al.’s study included only 23 PPMS patients, who were diagnosed according to the diagnostic criteria specified by Poser. We did not find any positive correlations between EDSS$_{LP}$ or disease progression rates and intrathecal IgG synthesis, except in the PPMS$_{TN}$ group. However, because of the small number of patients in this group, these results should be evaluated with caution.

The MRZ reaction was positive in slightly more than half of the PPMS patients, a slightly lower percentage than what was observed in clinically isolated syndrome (CIS) and RRMS 60–70% (Reiber et al. 1998, Brettschneider et al. 2009, Jarius et al. 2017) and is similar to a recent report from one of the study centres (Hottenrott et al. 2017). The difference might reflect lower prevalence of polyspecific immunoglobulin synthesis in PPMS or may be explained by various assays applied in different studies.

Data reports on the prevalence of intrathecal IgM synthesis in PPMS are scarce. We only found one such study, which reported elevated IgM index in
32.3% of its 29 patients with chronic progressive MS (Reboul et al. 1995). Increased IgM-index values might include false-positive results due to the linear formula used. Likewise, no studies investigating the intrathecal IgA synthesis in PPMS have been published thus far.

For the first time, a negative correlation between absolute levels of intrathecally produced IgM and IgA in CSF and disease progression has been reported in a large PPMS cohort. The correlation we found might indicate a possible protective role (e.g., anti-inflammatory or remyelinating) for IgM and IgA in PPMS. This role, postulated from in-vitro results, may be explained by the stimulatory effects of IgM on the oligodendrocytes as well as axonal protection (Warrington et al. 2010, Wootla et al. 2016).

By using the IgX index and the Reiber formula to calculate the absolute amounts of intrathecal synthesis of immunoglobulins in mg/l, it becomes clear that the discrepancies among the prevalence of intrathecal IgG, IgM, and IgA synthesis are caused by the linear (IgX index) versus non-linear (IgXloc) relationships between the Q_{ALB} and Q_{IgX} (Reiber et al. 2011).

EDSS_{LP} and disease progression rate did not differ between treated and untreated PPMS patients. Additionally, we did not find any positive correlations between EDSS_{LP} or disease progression rate and intrathecal IgG synthesis; but a trend towards higher progression rate in patients with pattern
3 OCB in PPMS\textsubscript{TN}. This might indicate a role of systemic inflammation in PPMS.

Indeed, the presence of elevated different inflammatory mediators in serum of PPMS patients was reported in previous studies (McDonnell et al. 1999, Ukkonen et al. 2007) and a recent study showed prominent follicular T-Helper (T\textsubscript{FH}) and B cell activation in blood from progressive MS patients with a correlation to disease progression (Romme Christensen et al. 2013).

Apart from that, it is likely that PPMS patients are not a homogenous group regarding the underlying inflammation (Abdelhak et al. 2017), which is a relevant aspect regarding the choice of therapy. In the absence of systemic inflammation and given an intact BBB, an intrathecal therapy that mainly targets the inflammatory process in the CNS seems to be a more rational alternative than a systemic therapy, which would be associated with lower bioavailability in the CNS and possibly more systemic side effects. Whether or not patients with signs of systemic inflammation, such as pattern 3 OCB, may rather profit from a systemically applied therapy, remains an interesting open question.

Our study has some drawbacks; the retrospective study design, the incompleteness of clinical data, assessing the clinical severity using the EDSS score instead of the more accurate multiple sclerosis severity score (MSSS), the variable follow-up period and the absence of correlation with imaging
studies. However, the large number of patients in our cohort and the multicentric aspect of the design are major advantages of study, which may be the last one to include the treatment of naïve PPMS patients as different effective disease-modifying drugs are expected to enter the market in the near future (Helwick 2016, Tourbah et al. 2016).

The results of our study inspire further prospective studies to validate our results, assess the prognostic value of CSF lactate, better define the role of IgM and IgA synthesis. Moreover, our study established a Network between highly specialised MS-centers, which eases further cooperation projects. Indeed, more than one hundred paired CSF-serum samples from the 254 patients and currently being analysed allows an in-depth biomarker-based profiling of the PPMS patients, which of great importance to better understand the underlying complex pathophysiological aspects. Furthermore, our cohort represents a core for a prospective PPMS register, which is currently in the final stages of planning.

In summary, our study included the most detailed CSF results of the largest PPMS cohort hitherto reported, with 254 PPMS patients. The main findings were the following: a) the high diagnostic sensitivity of intrathecally produced OCBs, which mainly consisted of pattern 2; b) the positive correlation of CSF-lactate levels with clinical severity and yearly progression rates; and c) the
possible protective role of intrathecally synthesized IgM and IgA, which may be of therapeutic relevance.
5. Summary:
Primary progressive multiple sclerosis (PPMS) is a relatively rare form of multiple sclerosis. The diagnosis and treatment of PPMS are considered challenging due to different causes. One of them is the lack of a well characterized cerebrospinal fluid (CSF) profile. The published cohorts either suffer small sample size or reported incomplete data. We designed the largest multicentric retrospective cohort to define the CSF-profile in PPMS patients. Our cohort included 4 university hospitals in Germany and compromised 254 patients. The basic CSF parameters like cell count, albumin quotient and lactate did not deviate from normal references. The intrathecal synthesis of IgG was detectable in more than 90% of the patients highlighting the diagnostic importance of this test and the inflammatory nature of the disease. The isoelectric focusing was much sensitive detecting the intrathecally synthesised immunoglobulins than the quantitative measurement using the Reiber formula. We reported for the first time a positive correlation between the CSF lactate and the yearly progression rate in PPMS patients. Further prospective multicentric studies with a larger sample size would be helpful to confirm our findings.
6. References:


Lebenslauf

Aus Datenschutzgründen entfernt