

Results of cerebrospinal fluid analysis in 108 patients with progressive multifocal leukoencephalopathy

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Research

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Abstract

Background: Progressive multifocal leukoencephalopathy (PML) is caused by an opportunistic infection with JC polyoma virus (JCPyV) and mainly affects immunocompromised patients. It leads to pronounced demyelination of the central nervous system (CNS) resulting in severe disability or even death. Detection of JCPyV DNA in the cerebrospinal fluid (CSF) is usually accepted as proof for the diagnosis of PML. Values from routine CSF parameters, like CSF cell count, protein concentration, Qalbumin, or intrathecal immunoglobulin synthesis are mostly considered as normal. However, this has not been investigated systematically. **Methods:** We therefore analyzed those standard CSF parameters in a cohort of 108 PML patients that were treated at four different neurological centers in Germany. The patients exhibited different underlying conditions with natalizumab treatment in multiple sclerosis (n=54) and human immunodeficiency virus (HIV)-infection (n=25) being the most frequent. The data were collected at the respective centers in accordance with local requirements and then jointly analyzed. The results of the total PML cohort were compared with a control group of patients with normal pressure hydrocephalus (NPH) and idiopathic intracranial hypertension (IIH). Multiple sclerosis and HIV patients were additionally compared with their own non-PML control groups. **Results:** The PML group showed an elevated cell count ($p < 0.001$) compared to the control group, however, this effect was mainly driven by HIV-PML patients. This subgroup also demonstrated a significantly higher proportion of patients with a disturbed blood-CSF-barrier function. **Conclusions:** This comprehensive, retrospective study on CSF diagnostic results in PML patients provides insight into the CSF findings in patients with PML. It demonstrates that CSF changes in PML patients may be specific for the underlying condition that predisposes for the development of PML and thus data have to be interpreted in this context.

Background

Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system (CNS) caused by reactivation of JC polyoma virus (JCPyV) and finally leading to a predominant destruction of oligodendrocytes (Tan et al, *Lancet Neurol* 2010). JCPyV is an opportunistic pathogen. Thus, PML primarily occurs in immune-compromised patients. The first pathological description of this disease was in 1958, whereby the affected patient suffered from chronic lymphatic leukemia and Hodgkin's disease (Aström et al, *Brain* 1958). The first human immunodeficiency virus (HIV)-infected patient with PML was reported in 1982 (Berger et al, *J Neurovirol* 1998). PML is also a well-known serious adverse event in natalizumab-treated multiple sclerosis (MS) patients (Blankenbach et al, *Neurology* 2019; Ho et al, *Lancet Neurol* 2017). Other conditions predisposing to PML are hematologic malignancies, post-transplant immunosuppression, or other diseases requiring immunosuppressive/immunomodulatory drugs (such as rituximab, cyclophosphamide, methotrexate, dimethyl fumarate, ciclosporin, mycophenolate mofetil, or fingolimod) (Clifford et al, *Arch Neurol* 2011; Calabrese et al, *Ann Rheum Dis* 2008, Berger et al, *Ann Neurol* 2009). Only in few instances, no apparent immunosuppression can be found (Zucker et al, *J Neurovirol* 2018), albeit immune senescence may play a role in such cases. PML is a rare disease, but it is characterized by high mortality rates and long-term

neurologic morbidity. In order to ensure a validated PML diagnosis, clinical, imaging, and laboratory features are needed (Berger et al, Neurology 2013). The detection of JCPyV DNA in CSF in combination with appropriate clinical symptoms and radiological characteristics allow a definite diagnosis of PML without biopsy. In individual cases, i.e. if JCPyV DNA remains undetectable in CSF, the presence of characteristic pathoanatomic findings from a CNS biopsy specimen may be required to establish PML diagnosis (Berger et al, Neurology 2013). No evidence-based therapeutic options are available. Reconstitution of the immune system e.g. by withdrawal of immunosuppressive drugs or provision of antiretroviral therapy are the only therapies that have demonstrated survival benefit (Aksamit, Curr Treat Options Neurol 2008). More recently, favorable outcomes have been reported in PML patients treated with immune checkpoint inhibitor therapies (Cortese et al, NEJM 2019; Walter et al, NEJM 2019; Hoang et al, J Neurovirol 2019) and infusion of virus-specific T cells (Rezvani, Marin, NEJM 2019). Without doubt, an early diagnosis and consequently a prompt start of therapy/cessation of causal immune suppression could improve the prognosis. Unfortunately, the diagnosis of PML is often delayed. Potential reasons are the lack of specific radiographic features in brain magnetic resonance imaging (MRI) and the fact that CSF JCPyV-PCR may be negative, particularly at an early stage of the disease. The search for a potential blood- or CSF-biomarker for PML, especially in CSF negative patients, has not been successful so far (Möhn et al, Fluids Barriers CNS 2019). Generally, there is a fundamental lack of information on routine CSF results in PML patients. Even though large cohorts of PML patients under natalizumab treatment have been described (Bloomgren et al, NEJM 2012; Landi et al, Neurology 2017) and a large retrospective, observational study about risk factors and outcomes of PML patients has been published recently (Anand et al, Neurol Neuroimmunol Neuroinflamm. 2019), results about basic CSF parameters have not been published in detail. Especially in times of promising, new therapeutic options, knowledge about distinct diagnostic features of CSF analysis is important to define risk groups or to evaluate treatment response. Here, we report the results of routine CSF parameters of 108 PML patients that were treated at four University hospitals in Germany. CSF results were further linked to patients' clinical data and their JCPyV PCR results.

Methods

Study design and setting

Patients were treated at the Department of Neurology at Hannover Medical School (n=50), St. Josef Hospital Bochum (n=38), University Hospital Münster (n=19), and University Hospital Cologne (n=12). Five patients who suffered from HIV infection were excluded for further analysis because three of them were diagnosed with additional cerebral toxoplasmosis and in two patients PML diagnosis was only suspected. Another six patients had to be excluded due to insufficient data quality, resulting in n=108 patients available for final analysis. In all patients that were diagnosed with PML other viral CNS infections such as varicella zoster virus (VZV)-, herpes simplex virus (HSV)-, Epstein-Barr virus (EBV)-, or cytomegalovirus (CMV) encephalitis had been ruled out. Results of the PML cohort were compared with an age-matched control group consisting of patients with normal pressure hydrocephalus (NPH) (n=8)

and idiopathic intracranial hypertension (IIH) (n=13) (suppl. table 2). In addition, the data of the HIV subgroup were compared with an HIV control group (n=37) whose patients had undergone a lumbar puncture for other reasons, for example cognitive deficits, suspected encephalitis, suspected vasculitis, unexplained encephalopathy, or seizures (suppl. table 3).

Diagnostic procedures

CSF and serum were analyzed by routine methods (Skrípuletz et al, Dis Markers 2014). CSF cell count, total protein, and lactate were analyzed immediately after CSF withdrawal by lumbar puncture. CSF cells were counted manually with a Fuchs-Rosenthal counting chamber. For further analyses the residual CSF was centrifuged (145 g for 15 min) and the supernatant frozen at -70°C . The function of the blood-CSF barrier was estimated as CSF/serum albumin quotients (Qalbumin). The age-adjusted upper reference limit of Qalbumin (Qalb) was calculated using the formula $Qalb = 4 + (\text{age in years}/15)$ (Reiber, Mult Scler 1998). CSF oligoclonal bands were determined by isoelectric focusing in polyacrylamide gels with consecutive silver staining. Five patterns of oligoclonal bands were distinguished following the recommendations of the first European consensus on CSF analysis in multiple sclerosis (Andersson et al, J Neurol Neurosurg Psychiatry 1994).

Statistical analysis

Statistical analysis was performed using GraphPad Prism software version 8.0 (GraphPad Software, San Diego, USA). The Mann-Whitney U-test as the nonparametric equivalent of the t-test for independent samples was used to compare the individual groups. For this test the data does not need to be normally distributed, the variables only need to be ordinally scaled. Data are displayed as means and standard error of the mean (SEM) or standard deviation (SD), respectively. $P < 0.05$ was considered statistically significant.

Results

Patients characteristics and standard CSF parameters

In total, the CSF results of 108 patients from four centers were analyzed. As many patients received several lumbar punctures, a total of 316 CSF analyses were considered. The patients' mean age was 48 years, with a range from 19 to 81 years. The underlying diagnoses included multiple sclerosis treated with natalizumab (MS/NTZ, n = 54), HIV infection (n = 25), and hematological diseases such as B-cell non-Hodgkin lymphoma (n = 10), multiple myeloma (n = 2), chronic lymphocytic leukemia (n = 6), and acute myeloid leukemia (n = 1) (referred to collectively as lymphoma group). Ten patients of the lymphoma group were treated with rituximab mono- or combination-therapy. Other chemotherapy regimens included bendamustine, melphalane, mitoxantrone, or methotrexate. In four of the patients an organ transplant had been performed and they received immunosuppressive therapy with tacrolimus, mycophenolate

mofetil, or ciclosporin. One patient each suffered from bronchial carcinoma, sarcoidosis, microscopic polyangiitis and common variable immunodeficiency (CVID). In two cases no explanatory underlying disease was found. Detailed information about the individual patients can be found in supplementary table 1. Regarding the standard CSF parameter of the first lumbar puncture (Table 1), 24/108 patients (22%) had an elevated cell count and 35/108 patients showed an elevated Qalbumin indicating a disturbed blood-CSF-barrier. The mean lactate content was 1.64 mmol/l (range: 0.99–2.8 mmol/l), whereby 6/108 (6%) subjects presented with increased lactate levels.

Table 1

Standard CSF parameters of first LP (whole cohort). CSF: cerebrospinal fluid. *With an average age of the cohort of 48 years, the upper limit of Qalbumin is 7.2 (Qalbumin = 4 + (age/15)).

	Standard value	Elevated (n)	Mean	Maximum	Minimum
Cell count (cell/ μ l)	≤ 4	24	5	34	1
Qalbumin	$< 7.3^*$	35	8.62	25.1	1.9
CSF protein (mg/l)	≤ 500	41	541	1330	226
CSF lactate (mmol/l)	< 2.2	6	1.64	2.8	0.99

Comparison of PML cohort and control group

Standard CSF parameters of the whole PML cohort were compared with CSF results of an aged matched control group (suppl. table 2) consisting of patients with NPH (n = 8) or IIH (n = 13). The mean age of the control group was 50 years. In the case of CSF lactate, Qalbumin, and CSF protein there was no difference between the two groups. In contrast, the CSF mean cell count of the PML cohort was higher compared with the control group (Fig. 1).

Comparison of different groups regarding standard CSF parameters at first lumbar puncture

We then compared the PML patients with different underlying diagnoses (Fig. 2). Patients with PML due to HIV infection had a higher cell count, CSF protein, Qalbumin, and CSF lactate compared with MS patients who suffered from PML because of natalizumab treatment. Also, in comparison with the control group, the HIV PML patients showed a higher cell count, CSF lactate, CSF protein, and Qalbumin level. Other PML subgroups compared among each other and in comparison with the control group had similar standard CSF parameter.

Comparison between HIV control group and HIV-PML group

To investigate whether the differences between HIV-PML patients and the other subgroups regarding standard CSF parameters were caused by either the HIV infection itself or by the PML, the HIV-PML patients were compared with an HIV control group without PML (suppl. table 3). While there was no significant difference concerning CSF lactate, HIV-PML patients showed a significantly higher CSF cell count, CSF protein and Qalbumin compared with the

non-PML control group (Fig. 3).

Analysis of standard CSF parameters in subsequent lumbar punctures

Several patients received subsequent lumbar punctures after PML was initially diagnosed. The maximum number of lumbar punctures in one patient was 26. To investigate whether the results of CSF analysis changed over time, the average time between the first lumbar puncture and subsequent punctures was calculated. In total, up to the first 9 punctures were considered. The results of the further punctures (10 to max. 26) were not considered representative for the total cohort because of too few patients. None of the parameters changed significantly over time or in the course of subsequent punctures (Fig. 4).

CSF cell distribution in PML patients at first lumbar puncture

In 85 patients of the total cohort a differentiation of cell distribution was performed at the first lumbar puncture. Sixty-seven patients (79%) showed a lymphocytic predominance while in 10 patients (12%) the majority of cells was monocytic. Six patients (7%) exhibited a mixed cell distribution and only two patients (2%) demonstrated mainly granulocytes within the CSF. The latter is best explained by artificial blood admixture. Considering the individual subgroups, patients of the MS/NTZ-, the HIV-, the lymphoma- and the transplant-group all showed a lymphocytic predominance (Table 2). Regarding both control groups, the clear majority of patients showed a lymphocytic predominance. A certain percentage of HIV patients showed a mixed cell distribution, whereby in all of those lumbar punctures an artificial blood admixture could be observed.

MS Group	HIV Group	Lymphoma Group	Transplant + Others Group	Control group	HIV control group
<ul style="list-style-type: none">• Lymphocytic predominance in 84%• Granulocytic predominance in 4%• Monocytic predominance in 4%• Mixed cell distribution in 8%	<ul style="list-style-type: none">• Lymphocytic predominance in 87%• Monocytic predominance in 6.5%• Mixed cell distribution in 6.5%	<ul style="list-style-type: none">• Lymphocytic predominance in 65%• Monocytic predominance in 29%• Mixed cell distribution in 6%	<ul style="list-style-type: none">• Lymphocytic predominance in 86%• Monocytic predominance in 14%	<ul style="list-style-type: none">• Lymphocytic predominance in 95%• Monocytic predominance in 5%	<ul style="list-style-type: none">• Lymphocytic predominance in 85%• Monocytic predominance in 3%• Mixed cell distribution in 12%

Table 2

CSF cell distribution of PML subgroups. HIV: human immunodeficiency virus, MS: multiple sclerosis. Normal cell distribution is defined as 90 – 60% lymphocytes and 10–40% monocytes.

	cell count (cell/ μ l)	Qalbumin	protein (mg/l)	lactate (mmol/l)	lymphocytes (%)	monocytes (%)	granulocytes (%)
only PML (n = 18)	1.4 \pm 2.7	6.07 \pm 2.91	499 \pm 469	1.58 \pm 0.76	88.8 \pm 23.1	18.8 \pm 31.1	9 \pm 9.2
IRIS (n = 26)	3.7 \pm 11.2	6.95 \pm 8.72	514 \pm 585	1.91 \pm 1.13	77 \pm 53.5	13.4 \pm 26.5	28.8 \pm 47.6
p-value	0.052	0.553	0.849	0.052			
Analysis of CSF parameters of patients who were diagnosed with PML-IRIS							

Table 3: Results of initial lumbar punctures compared with subsequent CSF analyses in patients with PML-IRIS. IRIS: immune reconstitution syndrome, PML: progressive multifocal leukoencephalopathy

In 18 patients of the total cohort (16 MS/NTZ patients, 2 HIV patients) detailed information about the occurrence of an immune reconstitution syndrome (IRIS) in subsequent lumbar punctures was available. Further 23 MS/NTZ patients presented with IRIS as well, but information about subsequent lumbar punctures was insufficient. A total of 26 additional lumbar punctures that were performed in PML-IRIS patients could be used for further analysis. Results of the initial CSF analyses (only PML) were compared with those of subsequent lumbar punctures (table 3). After IRIS was diagnosed, the patients tended to exhibit a higher cell count and higher lactate values. In contrast, no differences were observed regarding the other parameters. In addition, quantitative intrathecal immunoglobulin synthesis was determined in six IRIS-patients (data not shown). Two of them suffered from HIV infection as underlying diagnosis, the others belonged to the group of MS/NTZ patients. Except for one patient, all showed new intrathecal immunoglobulin synthesis during or after IRIS.

Comparison of CSF parameters of patients with JCPyV negative PCR with JCPyV PCR positive patients

Table 4

Comparison of JCPyV PCR positive patients and JCPyV negative patients at first LP. Neg: negative, PCR: polymerase chain reaction, Pos: positive.

	cell count (cell/ μ l)	Qalbumin	protein (mg/l)	lactate (mmol/l)
PCR neg (n = 19)	5	8.76	523.4	1.49
PCR pos (n = 76)	4.49	8.3	542.6	1.68
p-value	0.90	0.78	0.85	0.035

At the first lumbar puncture, the result of a JCPyV PCR analysis was available in 95 of 108 patients. Nineteen patients (20%) exhibited a negative JCPyV PCR while JCPyV PCR was positive in 76 patients (80%). While the comparison of both groups revealed no significant differences regarding CSF cell count, Qalbumin, or CSF protein, PCR positive patients exhibited a significant higher CSF lactate.

Analysis of oligoclonal bands of PML patients at first lumbar puncture

At first lumbar puncture oligoclonal bands (OCB) were analyzed in 58 patients. In 22 cases (38%) OCB type 2 (OCB in CSF only) were found, of which 18 patients belonged to the MS/NTZ group and one patient each to the HIV-, lymphoma-, and transplant-group. Nine patients (16%) exhibited OCB type 3 (identical OCB in CSF and serum and additional OCB in CSF only). The majority of patients (n = 5) suffered from HIV as underlying disease, two patients had MS/NTZ and one patient each belonged to the lymphoma and transplant group. Oligoclonal bands were negative in 27 patients, with 13 patients (22%) showing type 1 OCB (no OCB) and 14 patients (24%) showing type 4 OCB (identical OCB in CSF and serum) (Fig. 5). Seven patients (26%) of the MS/NTZ group exhibited negative oligoclonal bands at first lumbar puncture. This effect might be due to the natalizumab treatment which is known to modify oligoclonal bands (Mancuso et al, *Mult Scler* 2014; Harrer et al, *Mult Scler* 2013).

Discussion

Here, we present our results regarding the analysis of standard CSF parameters in a cohort of 108 PML patients. Compared with a control group consisting of patients diagnosed with NPH or IIH our cohort exhibited a significantly higher CSF cell count. However, this was mainly due to the HIV-PML group, while the CSF cell counts were normal in the other PML subgroups (Fig. 2). CSF protein and Qalbumin as an indicator for the integrity of the blood-CSF barrier function were similar in both PML and control groups (Fig. 1). Compared with the control group and the MS/NTZ-PML subgroup the HIV-PML patients had a higher CSF protein, Qalbumin, and CSF lactate (Fig. 2). It is likely that these inflammatory changes in HIV patients are due to the HIV infection itself. However, cell count of HIV-patients with validated PML was elevated in comparison with patients of the HIV control group (Fig. 3). Previous studies have shown that HIV infection is frequently accompanied by CSF pleocytosis occurring early in the infection and often resolving with antiretroviral therapy (Spudich et al, *J Infect Dis* 2006; Spudich et al, *BMC Infect Dis* 2005; Marshall et al, *Arch Neurol* 1988). A detailed analysis of the cellular composition of CSF in HIV-infected subjects via flow cytometry demonstrated that these patients exhibit an increase in the absolute number of CSF T cells and an even higher increase in the absolute number of CSF CD8 + T cells compared with a non-infected control-group (Ho et al, *PLoS One* 2013). The inflammatory processes in PML seem to lead to a further increase in CSF cell count in HIV patients and one can only speculate about the reasons for this. In addition to the cell count, protein and Qalbumin levels of the HIV-PML group were significantly elevated compared to the respective control group as well (Fig. 3). This indicates that the presence of PML in HIV patients leads to an increased dysfunction of the blood-CSF barrier. In summary, the majority of PML patients show inconspicuous results on CSF testing. In the subgroup of HIV patients, however, increased cell counts as well as elevated CSF protein and Qalbumin levels are regularly observed.

Pleocytosis may be an effect of the CNS involvement of the HIV infection itself, but just like CSF protein concentrations and Qalbumin levels, CSF cell count in HIV patients might also increase due to PML.

In the few patients of the cohort in whom IRIS was documented, a trend, albeit not statistically significant, for an increase in cell count and CSF lactate concentration was observed, while the other parameters remained unchanged (table 3). In addition, the immune response during IRIS seems to lead to augmented intrathecal immunoglobulin production (table 5). Previous pathological examinations have stated that the ratio of CD8 + cells to JCPyV infected cells is seventy times higher in cases of IRIS than in PML patients without an inflammatory response (Martin-Blondel et al, Neurology 2013). Pathologic observations in natalizumab associated PML emphasized the invasion of CD8 + cells as well as plasma cells with reduction of the viral load in case of IRIS (Metz et al, Acta Neuropathol 2012).

The detection of OCB at the initial lumbar puncture was highly dependent on the underlying disease. Thus, the majority of patients with intrathecal immunoglobulin synthesis belonged to the MS/NTZ-subgroup (Fig. 5). However, some patients of the other groups, especially HIV infected patients, exhibited positive OCB as well. This is consistent with the observation that HIV infection induces a humoral immune response in the CNS, as measured by an increased intrathecal IgG production, in both neurologically asymptomatic and symptomatic patients (Gisslén et al, Scand J Infect Dis 1994; Marshall et al, Arch Neurol 1988).

Our study is limited by its retrospective approach and the heterogenous clinical data from different institutions. Individual subgroups were partly small and especially patients of the HIV subgroup exhibited a certain heterogeneity regarding duration of HIV infection or antiretroviral therapy. Due to the nature of this investigation, interpreting causal relations is difficult. However, to our knowledge this is the largest study on routine CSF parameters in PML patients with different underlying conditions.

Conclusion

CSF analysis is routinely performed for the differential diagnosis of PML. The detection of JCPyV DNA in the CSF is the decisive step in the diagnosis of this disease. So far it was assumed that CSF routine parameter are normal in PML, however, our data show that an elevated cell count does not rule out PML, especially in patients with underlying HIV infection. These patients may also exhibit a disturbed blood-CSF barrier. Additionally, an increasing cell count and intrathecal immunoglobulin synthesis can indicate an immune reconstitution syndrome. However, immunoglobulin production and oligoclonal bands also depend on the underlying disease entity i.e. like a type 2/3 pattern in MS. Thus, CSF data in patients with suspected PML have to be carefully interpreted in the context of the underlying cause of the immunodeficiency, comorbidities and concomitant treatments.

Abbreviations

CMV

Cytomegalovirus
CNS
Central nervous system
CSF
Cerebrospinal fluid
EBV
Epstein-Barr virus
HIV
Human immunodeficiency virus
HSV
Herpes simplex virus
IIH
Idiopathic intracranial hypertension
IRIS
Immune reconstitution syndrome
JCPyV
JC polyoma virus
MRI
Magnetic resonance imaging
MS
Multiple sclerosis
NPH
Normal pressure hydrocephalus
PML
Progressive multifocal leukoencephalopathy
VZV
Varicella zoster virus

Declarations

Ethics approval and consent to participate

This study was approved by the local ethics committee (approval no. 2413 – 2014).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Figures

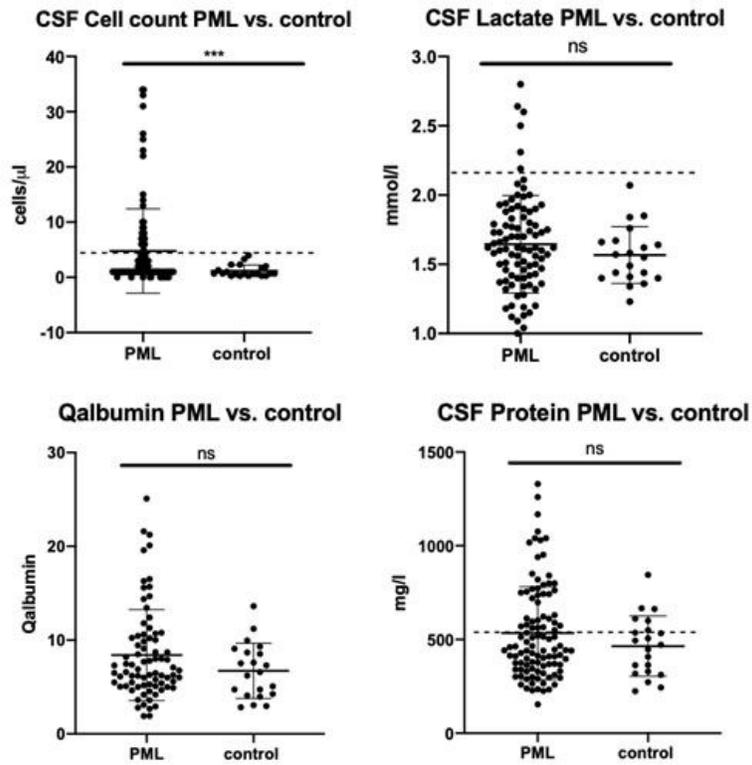


Figure 1

Comparison of CSF cell count, lactate, Qalbumin, and CSF protein of PML patients with control patients. PML: progressive multifocal leukoencephalopathy. Data is presented as mean \pm standard deviation. Levels of significance: *** $p < 0.001$, two-tailed p -value, Mann-Whitney-test with comparison of median values was applied. ns: not significant.

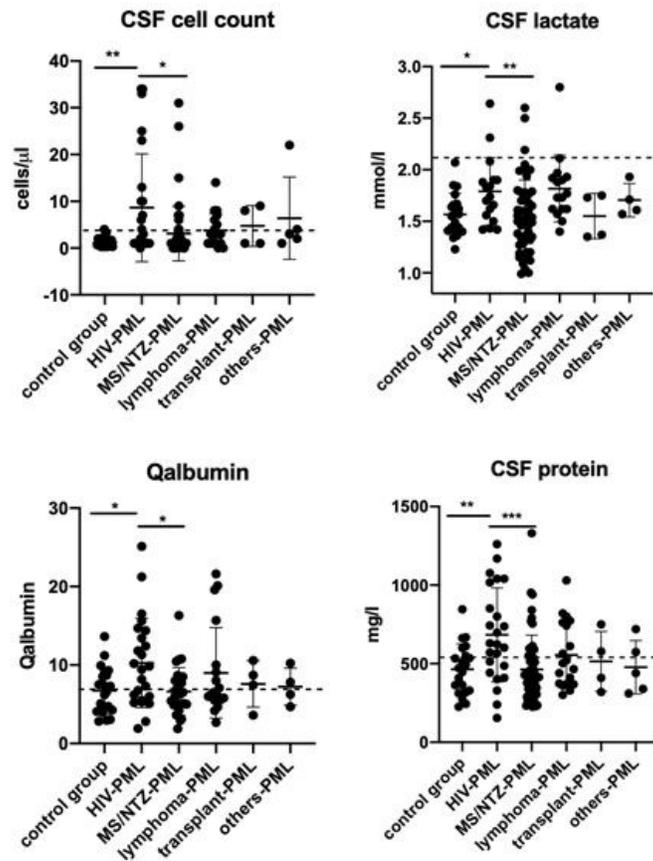


Figure 2

Comparison of different PML subgroups and control group regarding CSF cell count, Qalbumin, CSF protein, and CSF lactate. CSF: cerebrospinal fluid, HIV: human immunodeficiency virus, MS: multiple sclerosis. Data is presented as mean \pm standard deviation. Levels of significance: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, two-tailed p-value, Mann-Whitney-test with comparison of median values was applied.

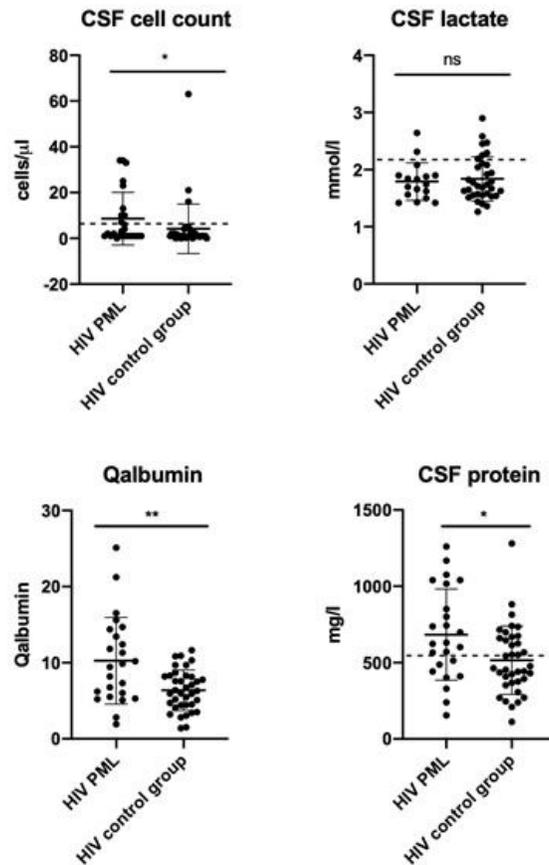


Figure 3

Comparison of HIV PML patients with HIV patients without PML regarding CSF cell count, CSF lactate, CSF protein and Qalbumin. CSF: cerebrospinal fluid, HIV: human immunodeficiency virus, PML: progressive multifocal leukoencephalopathy. Data is presented as mean \pm standard deviation. Levels of significance: ** $p < 0.01$, * $p < 0.05$, ns: not significant, two-tailed p-value, Mann-Whitney-test with comparison of median values was applied.

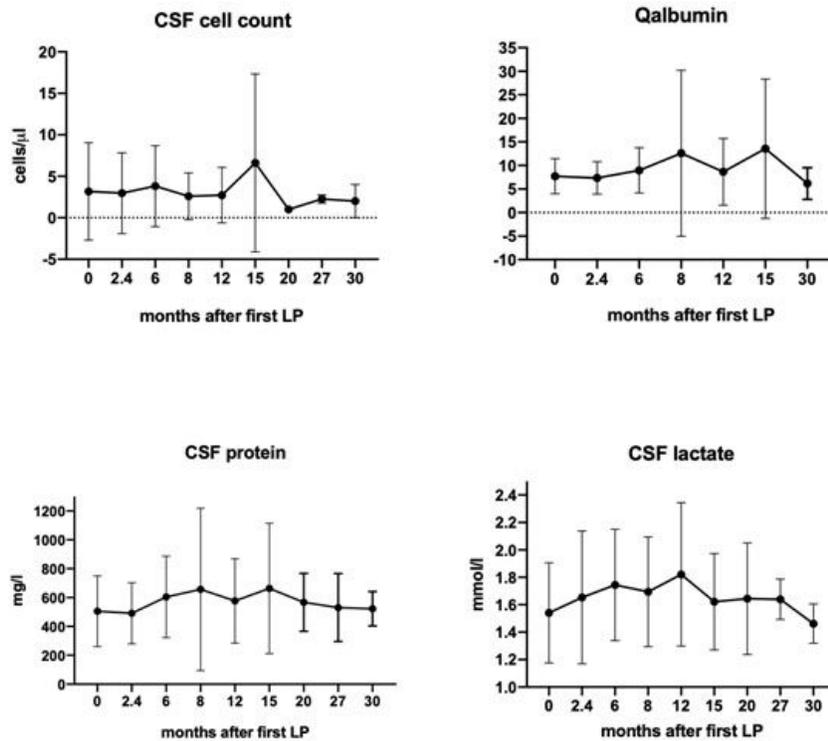


Figure 4

Presentation of standard CSF parameters in the course of the subsequent lumbar punctures. Data presented as mean \pm standard deviation. On the x-axis the figure shows the average time since first lumbar puncture (months). LP: lumbar puncture. 1. LP: n=55, 2. LP: n=43; 3. LP: n=30; 4. LP: n=19; 5. LP: n=12; 6. LP: n=7; 7. LP: n=6; 8. LP: n=5; 9. LP: n=4.

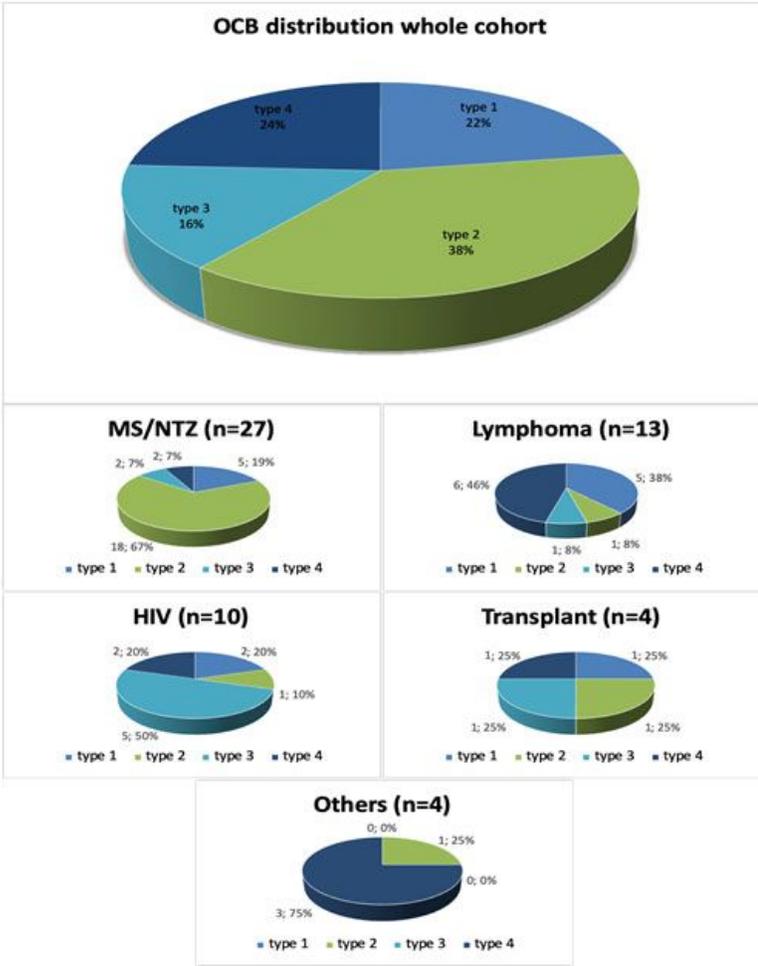


Figure 5

Analysis of oligoclonal bands (OCB) at first lumbar puncture. Type 2 and type 3 OCB indicate intrathecal immunoglobulin synthesis. HIV: human immunodeficiency virus, MS: multiple sclerosis, NTZ: natalizumab.