Glial Activation Markers in CSF and Serum From Patients With Primary Progressive Multiple Sclerosis: Potential of Serum GFAP as Disease Severity Marker?

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Background: In progressive multiple sclerosis (MS), glial activation is thought to be a relevant mechanism of disability progression. Therefore, in vivo assessment of the glial cell activity is, in the emerging treatment era of primary progressive MS (PPMS), more important than ever.

Objectives: To test the association of cerebrospinal fluid (CSF) and serum markers of glial activation in PPMS patients; including glial fibrillary acidic protein (GFAP), chitinase-3-like protein 1 (CHI3L1), soluble variant of triggering receptor expressed on myeloid cells 2 (sTREM2), and marker of neuroaxonal damage (Neurofilament light chain, NfL) as well as clinical severity.

Methods: CSF and serum samples from PPMS patients were collected in the MS-centers at Universities of Freiburg (n = 49), Ulm (n = 27), Muenster (n = 11), and Rostock (n = 6). sTREM2 and CHI3L1 levels were measured using the previously reported ELISA assays, while NfL and GFAP were measured using SIMOA assays. Clinical data included age, gender, disease duration, treatment status, and Expanded Disability Status Scale (EDSS).

Results: 93 CSF samples and 71 matching serum samples were analyzed. The median age of patients was 49 years and disease duration 4.5 years. GFAP serum correlated with EDSS after correction for age (β = 0.3, p = 0.001). Furthermore, EDSS was higher in patients with a GFAP serum level ≥ 151.7 pg/ml compared to patients with GFAP serum level below this cut-off (5.5 vs. 4.0, p = 0.009). Other markers did not correlate with the clinical severity. Moreover, we found a correlation between NfLCSF and GFAPCSF, sTREM2 and...
INTRODUCTION

The pathophysiology of primary progressive multiple sclerosis (PPMS) is complex and involves various mechanisms including inflammatory triggered demyelination, activation of B and T lymphocytes, mitochondrial dysfunction, and iron accumulation (1). However, the glial activation is considered to play a decisive role in the progression of neuroaxonal demise (2–4). While clinical and many radiological parameters can detect the final pathway of those different pathophysiological processes (progression of clinical disability, new MRI-lesions etc.), several other aspects such as role, extent and contribution of the various pathophysiological mechanisms remain widely unexplored. A biomarker-based approach may offer a unique window to assess such disease processes in vivo (5, 6). Over the last years, the level and clinical meaning of different biomarkers in CSF like glial fibrillary acidic protein (GFAP) as a marker for astrocytic activation (7–16), chitinase 3 like 1 protein (CHI3L1) (13, 14, 17–19) and soluble triggering receptor expressed on myeloid cells 2 (sTREM2) for microglial activation (20–23) and neurofilaments light chain (NfL) for neuroaxonal damage were reported. The single molecular assay (SIMOA) enables the detection of the ultra-low concentration of some of those biomarkers in serum (16, 24, 25). We previously showed that GFAP in serum correlates with the Expanded Disability Status Scale (EDSS) specifically in PPMS but not in patients with a relapsing-remitting disease course (16). Similar results were reported later from other groups (26). In this study, we aim to reproduce these findings in a large cohort of PPMS patients and to explore the clinical meaning of the other glial activation markers in PPMS.

METHODS

Patient Selection

CSF and serum samples from patients with PPMS were collected from the University Hospitals of Freiburg, Ulm, Muenster, and Rostock. The patients were admitted or seen within the Outpatient Departments between 2010 and 2018. In all patients, the diagnosis has been revised according to the McDonald criteria from 2017 (27) after careful exclusion of relevant differential diagnoses. The lumbar puncture was performed as a part of the diagnostic workup. The clinical severity was measured by assessing the Expanded Disability Status Scale (EDSS), Multiple Sclerosis Severity Score (MSSS) as well as the Age-related Multiple Sclerosis Severity Score (ARMSS) as reported recently (28).

CSF and Serum Sample Processing

A standardized protocol for CSF and serum collection was applied as previously recommended (29). Biosamples from patients were stored according to the predefined standard operating procedure (SOPs) at a local biobank at minus 80°C. Later they were transferred for measurement on dry ice to the biobank of the coordinating center in Ulm for further analysis. Hemolytic CSF specimens were excluded. From some patients, only CSF samples were available, with no matching serum samples.

Assessments of the Biomarkers

GFAP and NfL in CSF and serum were measured using Simoa assays (GFAP Discovery kits and NfL Early Access assays, Quanterix Corporation). CHI3L1 was measured using the commercial ELISA-Kits (Human Chitinase 3-like 1 Quantikine ELISA Kit DC3L10, R&D Systems). sTREM2 was measured using the previously reported ELISA using the MSD Platform (21). Samples were diluted, as recommended by the manufacturer, and concentrations were calculated using the corresponding standard curve. The intra-assay coefficient of variation (CV) was assessed by measuring a QC of serum and CSF sample in 5 replicates with a CV below 10% was obtained, whereas a CV of lower than 10% had to be achieved for a valid analysis. We did not find an influence of up to 5 freeze-thaw cycles on the investigated biomarkers, except for GFAP in CSF. Here, the concentration decreased by over 50% after within 2 freeze-thaw cycles. Therefore, GFAP CSF levels between centers were compared, and exceedingly low values were excluded from the analysis.

To compare potentially pathological serum biomarker levels, we determined a cut-off in a group of 20 patients with other non-inflammatory neurological diseases we previously published (16). As the concentration of serum GFAP in a normal or healthy population is not described, we used the 90th percentile to determine a cut-off value for further analysis. This yielded a cut-off value of 151.7 pg/ml for serum GFAP. A cut-off value for serum NfL of 16 pg/ml was suggested recently (30).
TABLE 1 | Clinical characteristics of the included subjects.

| Parameter                        | Median (25–75 percentile), n = 93 |
|----------------------------------|-----------------------------------|
| Age                              | 49 years (44–57)                  |
| Gender (♂/♀)                     | 1:1                               |
| Disease duration in years        | 4.5 (2–12)                        |
| Expanded disability status scale (EDSS) at the time of lumbar puncture (LP) | 4.5 (3.5–6.5) |
| Multiple sclerosis severity score (MSSS) | 8.1 (6.4–9.1) |
| Age-related multiple sclerosis severity score (ARMSS) | 6.2 (4.6–7.9) |

Statistical Analysis

All statistical tests were performed using SPSS® Statistics version 25 (IBM Corporation). The Shapiro-Wilk test was used to examine the distribution of the data. Mann-Whitney U test and Kruskal-Wallis test were used to compare medians in skewed distributed parameters. A multiple linear regression model and univariate general linear model was applied to account for a possible confounding bias caused by the strong correlation between GFAP levels and age. The Spearman’s rho test was used to test for correlations. A univariant general linear model was applied to account for the effect of age on the grouping by GFAP level. Figures were made using GraphPad Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Clinical Characteristics

A total of 93 CSF and 71 matching serum samples were collected. The summary of the clinical characteristics is mentioned in Table 1, and the concentration of the different CSF and serum biomarkers are shown in Table 2. Fifty-five patients did not receive any disease modifying treatment at and before the time of lumbar puncture. Thirty-eight patients were on a treatment: Mitoxantrone (n = 19), three monthly pulse-steroid (n = 11), Rituximab (n = 5), cyclosporine (n = 2), and Interferon beta 1a (n = 1).

Comparison Between Centers

Despite having similar patients characteristics (age, gender distribution, disease duration, and disease severity as assessed by EDSS), GFAP(CSF) levels differ between the centers; values from Muenster and Rostock were significantly lower than those from Ulm and Freiburg, whereas sTREM2 levels were lower only in the samples from Rostock compared to all other centers (Figure 1). Thus, in the following analysis, we excluded the GFAP(CSF) from Muenster and Rostock (n = 17), and sTREM2 measurements from Rostock (n = 6). Concentrations of CHI3L1, NfL(CSF), NfL(serum), and GFAP(serum) in the samples of Muenster and Rostock were included in the statistical analysis.

TABLE 2 | Concentrations of the assessed biomarkers in CSF and serum.

| Biomarker                                        | Median (25–75 percentile) |
|--------------------------------------------------|---------------------------|
| Cerebrospinal fluid gial fibrillary acidic protein (GFAP(CSF)) | 7,820 (5,050–1,1165) |
| Serum gial fibrillary acidic protein (GFAP(serum)) | 126.0 (104.5–174.0) |
| Cerebrospinal fluid neurofilaments light chain (NfL(CSF)) | 1230.8 (840–2,125) |
| Serum neurofilaments light chain (NfL(serum)) | 18.5 (12.3–25.9) |
| Cerebrospinal fluid chitinase 3 like 1 protein (CHI3L1) | 210.8 (138.5–291.0) |
| Cerebrospinal fluid soluble triggering receptor expressed on myeloid cells 2 (sTREM2) | 3.1 (2.3–4.4) |

Clinical Aspects

None of the biomarkers correlated with the age except GFAP(serum) and NfL(serum) (ρ = 0.4 and 0.3, p = 0.005 and 0.014, respectively) (Figure 2). Gender and treatment status did not influence the levels any of the tested markers neither in CSF nor in serum (data not shown).

Of all the assessed markers (in CSF and in serum), only GFAP(CSF) correlated with disease duration (ρ = 0.3, p = 0.014).

None of the CSF markers correlated with disease severity.

Regarding the serum markers, we found a moderate correlation between GFAP(serum) and EDSS (ρ = 0.4, p = 0.004), which remained significant after adjusting by the effect of age (β = 0.3, p = 0.001) (Figure 3). NfL(serum) did not correlate with any of the disease severity parameters (Figure 3). No significant correlations were found between GFAP(serum) and MSSS or ARMSS (data not shown).

To further confirm the association of GFAP(serum) with the EDSS, we compared the EDSS values with GFAP(serum) and NfL(serum) levels higher or lower than the cut-off that was determined as described beforehand. Here, 63% of our PPMS patients had GFAP(serum) above this cut-off of 151.7 pg/ml (n = 45). Moreover, they had a significantly higher median EDSS than patients below this cut-off (5.5 vs. 4.0, p = 0.009, Figure 4). No differences were found for this comparison for NfL(serum) (cut-off 16 pg/ml, 4.5 vs. 4.5, p = 0.16, Figure 4). There was a significant age difference for the grouping by NfL(serum) but not for GFAP(serum) (p = 0.01 and 0.47, respectively, data not shown).

Considering NfL(serum) ≥ 16 pg/ml as a cut-off value for active diseases, PPMS patients with NfL(serum) ≥ 16 pg/ml (n = 44) have higher median concentration of GFAP(serum) than those with NfL(serum) < 16 pg/ml (n = 27) (131.0 vs. 114.5 pg/ml, p = 0.037 after correction for age, Figure 5).

Correlation Between CSF and Serum Parameters

We found a moderate correlation between GFAP levels in CSF and serum (ρ = 0.4, p = 0.001), and strong correlation between levels of NfL in CSF and serum (ρ = 0.6, p < 0.001).
In CSF, NfL correlated with sTREM2 ($\rho = 0.4, p < 0.01$), CHI3L1 ($\rho = 0.3, p < 0.01$) and GFAP ($\rho = 0.4, p < 0.001$, Table 3 and Figure 6). Moreover, CHI3L1 correlated with sTREM2 ($\rho = 0.4, p < 0.01$, Figure 7) but not with GFAP. On the other hand, GFAP did not correlate with sTREM2.
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FIGURE 4 | Comparison of expanded disability status scale (EDSS) values for primary progressive multiple sclerosis (PPMS) patients below and above cut-offs of 151.7 pg/ml for glial fibrillary acidic protein (GFAP) (A) and 16 pg/ml for neurofilaments light chain (NfL) (B) in serum (Mann-Whitney U test), respectively. There was a significant age difference for the grouping by NfL but not for GFAP (Mann-Whitney U test, p = 0.01 and 0.47, respectively). LP, lumbar puncture.

FIGURE 5 | Level of serum glial fibrillary acidic protein (GFAPserum) in PPMS patients with serum neurofilaments light chain levels equal to or higher than 16 pg/ml (NfLserum ≥ 16) or lower the 16 pg/ml (NfLserum < 16), *Univariant general linear model corrected for age.

TABLE 3 | Correlations between various CSF biomarkers.

| Marker     | NfLCSF | GFAPCSF | CHI3L1 |
|------------|--------|---------|--------|
| sTREM2     | 0.4**  | n.s.    | 0.4**  |
| CHI3L1     | 0.3**  | n.s.    |        |
| GFAPCSF    | 0.4*** |         |        |

GFAP, glial fibrillary acidic protein; NfL, neurofilaments light chain; CHI3L1, chitinase 3 like 1; sTREM2, soluble triggering receptor expressed on myeloid cells 2. **p < 0.01, ***p < 0.001.

DISCUSSION

Assessment of various biomarkers reflecting different pathophysiological processes involved in the disease progression and the downstream-treatment effect is becoming increasingly important due to the emerging treatment options for progressive MS. Our study evaluates the levels of astroglial activation (GFAP), microglial activation (CHI3L1 and sTREM2) and neuroaxonal damage (NfL) in CSF and serum of a multicentric cohort of PPMS-Patients.

The glial activation is a putative cornerstone in the progression of neurodegeneration in PPMS (1). The astrocytes involvement in MS generally is a double-edged sword; while the glial scar formation may protect the tissue from further damage, it might prevent the remyelination in MS (31). The activation of astrocytes is an early event in the development of MS lesions with the release of cytokines like CCL-2, CXCL-12, MMPs, TGFβ, IL-1, IL-6, IL-10, IL-12, IL-15, IL-23, and IL-27 leading to dysfunction of the blood-brain barrier (BBB) as well as recruitment of innate and adaptive immune cells (32). Furthermore, the A1 subtype of astrocytes is a potent killer of neurons and oligodendrocytes in EAE models and was reported in acute and chronic MS lesions (33).

Nevertheless, the astrocytes in progressive MS play a more specific role in the maintenance of the local inflammation; in models of chronic EAE, levels of lactosylceramide (LacCer) synthesized by β-1,4-galactosyltransferase 6 (B4GALT6) from the activated astrocytes were elevated in the EAE-lesions. Suppression of B4GALT6-activity reduces local inflammation, microglial activation and monocytes recruitment and subsequently the resulting neurodegeneration (34).

Correlation between GFAPserum and clinical severity were inconsistent among previous studies using the standard ELISA assay (9, 12–15). In a previous study from one of our centers, we reported a moderate correlation between levels of GFAP in serum, but not in CSF, with disease severity scores (16). Our current multicentric cohort validated our results showing a consistent correlation between GFAPserum with EDSS score, even after correction for the age of the patients. In accordance with those results, patients with GFAPserum above the
proposed cut-off of 151.7 pg/ml based on our previous results, had more severe disease. Furthermore, levels with GFAP_{serum} were higher in patients having NfL_{serum} ≥ 16, a recently proposed cut-off value for higher activity and worse disease prognosis (30). Nevertheless, this NfL cut-off value was proposed according to pooled results from various studies with relapsing-remitting MS patients. Thus, its value in PPMS has yet to be validated.

Why GFAP_{serum} might reflect the disease activity better than GFAP_{CSF} is still not entirely explained. As previously suggested from our group, the enhanced expression of GFAP in the activated astrocytes end feet in the predominantly perivascular MS lesions might be directly drained into the blood compartment and not into the CSF space (16). Supporting data were reported in mouse models of EAE (35). Moreover, the GFAP, as well as other markers, might be transported to blood directly via the glymphatic system as shown recently in murine models of traumatic brain injury (36).

While a recent study suggested lower levels of GFAP_{serum} in relapsing MS patients under treatment (26), the concentration of GFAP_{serum} did not differ according to treatment status in our cohort. This can be explained by the fact, that none of the above-mentioned treatments were proven effective in PPMS. This observation regarding levels of GFAP_{serum}, as well as other markers including the NfL_{serum}, might underscore the ineffectiveness of those treatments in PPMS.

The validation of those results in our larger multicentric cohort might highlight the clinical meaning of levels of GFAP in serum as a possible serum marker in PPMS patients.

Like the astrocytes, the activated microglial release different cytokines (IL-1, IL-6, and TNF-α) as well as NO and ROS leading to exacerbation of inflammatory cascade, to attraction of inflammatory cells from blood and also to mitochondrial dysfunction, a significant mechanism in the disease progression in PMS (2, 3, 37, 38). Moreover, microglial activation in PMS appears to be diffusely prevalent not only in MS lesions but also in normal-appearing white matter (NAWM) forming the so-called microglial nodules. In the less inflammatory cortical lesions, active demyelination can be found in close proximity with the microglia (3).

Data regarding sTREM2 in PPMS are scarce with two studies reported concentration in CSF in twenty-one and in three PPMS patients, respectively (20, 22). In accordance with previous reports, no correlation was found between sTREM2 and EDSS or MSSS. Yet, sTREM2 correlated with NfL in CSF, which might highlight the role of microglia in the neuroaxonal demise in PPMS.

The meaning of CHI3L1 appears to be controversial; some reports consider it as a marker of astrocytic activation (18, 39), whereas other studies count it a marker of active microglial cells (40). The correlation between CHI3L1 and sTREM2 found in our patients is equivocal; it may underscore the microglial origin of CHI3L1 or might reflect the crosstalk between the microglia and astrocytes in PPMS (33) leaving the question regarding the origin of CHI3L1 in MS brains unanswered.

The prognostic value of CHI3L1 is prominent in CIS and RRMS patients (13, 17, 41–44) and to a lesser extent in SPMS (19). CHI3L1 in CSF did not correlate with the clinical parameters in PPMS patients in the above-mentioned studies. Consistent with the various histopathological studies, all measured glial activation markers in CSF correlated with the neuroaxonal demise as assessed by the NfL, but not with the clinical severity scores. This paradox might be due to some methodological limitations of the assays, the need of more specific markers or due to the limited ability of the applied clinical scores to reflect the extent of the neurodegeneration in PPMS. Indeed, the shortness of EDSS to reflect all aspects of the disease progression in PPMS is a lesson learned from the various negative clinical trials in PPMS (45). The upper arm function and the subtle cognitive deficits are underrepresented in the EDSS (46). Furthermore, the EDSS scores were mostly based on the walking distance reported by the patients, which might lead to an incorrect evaluation of the EDSS score (47).

Another limitation of our study is the missing detailed magnetic resonance imaging data (MRI). However, the main aim of our study was to explore the clinical meaning of the glial activation markers.
Although the concentration of most of the measured biomarkers did not vary between centers, GFAP and sTREM2 were notably lower in two of the four centers. As this is a retrospective study, we were not able to completely capture all pre-analytical procedures. To avoid potential bias of pre-analytical procedures, we did the comparison of analyte levels per center and excluded statistically low values. The excluded samples represent a minority in our cohort (17/93 for GFAP and 6/93 for sTREM2), and their exclusion had no statistical impact on the above-mentioned results (data not shown).

A point to be considered while interpreting our results is the protective role of glial cells following neuronal injury. Microglial and astrocytic activation were reported upon axonal degeneration and contribute to tissue repair and limitation of the inflammatory activity (48–50). Our markers reflect the glial activation generally, but not necessarily their pathological role. Complementary data from more specific markers of subsets of microglia or astrocytes could be helpful to understand their pathogenetic role.

In summary, we analyzed in this study various markers of glial activation in CSF and serum and evaluated their correlation with neuroaxonal damage markers and disease severity measures. The correlation between the microglial and astrocytic activation markers in CSF with the markers of neuroaxonal demise (NfL) may underscore the glial involvement in the neurodegeneration in PPMS. The positive correlation between GFAP in serum with the clinical severity of the disease may highlight the potential of GFAPserum as a disease progression marker. It is highly desirable to confirm this finding in a prospectively collected study cohort and compare it to standardized acquired MRI data. Additionally, the determination of GFAP serum levels in a large group of healthy controls might help to further differentiate between age related normal and abnormal GFAP levels.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The study was reviewed by the ethics committee of the University of Ulm, and all experimental protocols were approved (approval number 270/17). Our study was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. Written informed consent was obtained from all patients participating in this study.

AUTHOR CONTRIBUTIONS

AA, HT, and AH: study concept. AA and TH: data acquisition, data analysis, and interpretation. AH, EM-R, and MS-C: biomarkers assessment. AA: drafting of the manuscript. HT, SR, MO, CH, UZ, and SM: study supervision and critical revision. All authors critically reviewed and approved the manuscript.

FUNDING

This study was supported in part by the German Federal Ministry of Education and Research (KKNMS and FTDLC), JNPD networks PreFrontAls (01ED1512), the EU (FAIR-PARK II), the German Research Foundation/DFG (SFB1279), the foundation of the state Baden-Württemberg, DFG funded Koselleck Project (HA1737/16-1 to CH), and the FTD Biomarker Award (to CH and MS-C).

ACKNOWLEDGMENTS

We acknowledge support by Deutsche Forschungsgemeinschaft and Open Access Publishing Fund of University of Tübingen.
12. Axelsson M, Malmström C, Nilsson S, Haghighi S, Rosgren L, Lycke J. Gliarial fibrillary acidic protein: a potential biomarker for progression in multiple sclerosis. J Neurol. (2011) 258:882–8. doi: 10.1007/s00415-010-5863-2
13. Martinez MA, Olsson B, Lau I, Matas E, Cobo Calvo A, Andreasson U, et al. Giall and neuronal markers in cerebrospinal fluid predict progression in multiple sclerosis. Mult Scler. (2015) 21:530–61. doi: 10.1177/1352458514539979
14. Mane-Martinez MA, Olsson B, Lau I, Matas E, Cobo Calvo A, Andreasson U, et al. Giall and neuronal markers in cerebrospinal fluid in different types of multiple sclerosis. J Neuroimmunol. (2016) 299:112–7. doi: 10.1016/j.neuroimmunol.2016.08.004
15. Kassubek R, Gorges M, Schocke M, Hagenston VAM, Huss A, Ludolph AC, et al. Serum GFAP as a biomarker for disease severity in multiple sclerosis. Sci Rep. (2018) 8:14798. doi: 10.1038/s41598-018-33158-8
16. Hinsinger G, Galeotti N, Nabhoez N, Urbach S, Rigau V, Demattei C, et al. Chitinase 3-like proteins as diagnostic and prognostic biomarkers of multiple sclerosis. Mult Scler. (2015) 21:1251–61. doi: 10.1177/1352458514561906
17. Querol-Vilaseca M, Colom-Cadena M, Pegueroles J, San Martin-Panillo C, Clarimon J, Belbin O, et al. YKL-40 (Chitinase 3-like 1) is expressed in a subset of astrocytes in Alzheimer’s disease and other tauopathies. J Neuroinflammation. (2017) 14:118. doi: 10.1186/s12974-017-0893-7
18. Burman J, Raininko R, Blennow K, Zetterberg H, Axelsson M, Malmstrom C, et al. Serum neurofilament light chain in early relapsing remitting MS is associated with neuronal injury markers. J Neuroimmunol. (2018) 324:186. doi: 10.1016/j.jneuroim.2018.07.004
19. Piccio L, Buonsanti C, Cellia M, Tassi I, Schmidt RE, Fenoglio C, et al. Identification of soluble TREM-2 in the cerebrospinal fluid and its association with multiple sclerosis and CNS inflammation. Brain. (2008) 131 (Pt 11):3081–91. doi: 10.1093/brain/awn217
20. Kleinberger G, Yamanishi Y, Suárez-Calvet M, Czirr E, Lohmann E, Cuyvers E, et al. TREM2 mutations implicated in neurodegeneration impair cell surfate transport and phagocytosis. Sci Transl Med. (2014) 6:243ra86. doi: 10.1126/scitranslmed.3009093
21. Ohrfelt A, Axelsson M, Malmoström C, Novakova I, Lesregaev A, Blennow K, et al. Soluble TREM-2 in cerebrospinal fluid from patients with multiple sclerosis treated with natalizumab or mitoxantrone. Mult Scler. (2016) 22:1587–95. doi: 10.1177/1352458516624588
22. Suarez-Calvet M, Kleinberger G, Araque Caballero MA, Bredel M, Rominger A, Alcolea D, et al. sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer’s disease and associate with neuronal injury markers. EMBO Mol Med. (2016) 8:466–76. doi: 10.15252/emmm.201506123
23. Kuhle J, Barro C, Disanto G, Mathias A, Soneson C, Bonnier G, et al. Serum neurofilament light chain in early relapsing MS is increased and correlates with CSF levels and with MRI measures of disease severity. Mult Scler. (2016) 22:1550–9. doi: 10.1177/1352458516623365
24. Bogoslovsky T, Wilson D, Schag H, Gill J, Jermol A, et al. Increases of Plasma Levels of Gliarial Fibrillary Acidic Protein, Tau, and Amyloid beta up to 90 Days after Traumatic Brain Injury. J Neurotrauma. (2017) 17:026–173. doi: 10.1016/j.jneurotrauma.2017.03.060
25. Manouchehrinia A, Westerlund H, Kingwell D, Zhu F, Carruthers R, Ramanuam R, et al. Age-related multiple sclerosis severity score: disability ranked by age. Mult Scler. (2017) 23:1938–46. doi: 10.1177/1352458517760968
26. Teunissen CE, Petzold A, Bennett JL, Berven FS, Brundin L, Comabella M, et al. A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. Neurology. (2009) 73:1914–22. doi: 10.1212/WNL.0b013e3181c74c2c
27. Calabresi P, Kuhle J, Arnold DL, Singh CM, Kinkel RP, Kappos L, et al. Serum Neurofilament Light (NFL) for Disease Progression and Treatment Monitoring in Multiple Sclerosis Patients: Is It Ready for Implementation into Clinical Care? Berlin: European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) (2018).
28. Holley JE, Gveric D, Newcombe CJ, Cuzner ML, Gutowski NJ. Astrocyte characterization in the multiple sclerosis glial scar. Neurapathol Appl Neurop. (2003) 29:434–44. doi: 10.1046/j.1600-2990.2003.00491.x
29. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. Nature. (2017) 541:481–7. doi: 10.1038/nature21029
30. Mayol L, Trauger SA, Blain M, Nadeau M, Patel B, Alvarez JL, et al. Regulation of astrocyte activation by glycolipids drives chronic CNS inflammation. Nat Med. (2014) 20:1147–56. doi: 10.1038/nm3681
31. Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. Acta Neuropathol. (2010) 119:7–35. doi: 10.1007/s00401-009-0619-8
32. Michelucci A, Heurtaux T, Grandbarbe L, Morga E, Heuschling P. Characterization of the mioglobin phenotype under specific pro-inflammatory and anti-inflammatory conditions: effects of oligomeric and fibrillar amyloid-beta. J Neuroimmunol. (2009) 210:3–12. doi: 10.1016/j.jneuroim.2009.02.003
33. Comabella M, Fernandez M, Martin R, Rivera-Valle S, Borras E, Chiva C, et al. Cerebrospinal fluid chitinase 3-like 1 levels are associated with conversion to multiple sclerosis. Brain. (2010) 133 (Pt 4):1082–93. doi: 10.1093/brain/awp035
34. Sellebjerg F, Royen L, Soelberg Sorensen P, Oturai AB, Jensen P EH. Biomarkers of trauma are transported from brain to blood via the glymphatic system. Brain. (2018) 141:2090–13. doi: 10.1093/brain/awx113
35. Holley JE, Gveric D, Newcombe CJ, Cuzner ML, Gutowski NJ. Astrocyte characterization in the multiple sclerosis glial scar. Neurology. (2017) 541:481–7. doi: 10.1038/nature21029
36. Bogoslovsky T, Wilson D, Schag H, Gill J, Jermol A, et al. Increases of Plasma Levels of Gliarial Fibrillary Acidic Protein, Tau, and Amyloid beta up to 90 Days after Traumatic Brain Injury. J Neurotrauma. (2017) 17:026–173. doi: 10.1016/j.jneurotrauma.2017.03.060
37. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. (2017) 16:606–617. doi: 10.1016/S1474-4422(17)30470-2
38. Manouchehrinia A, Westerlund H, Kingwell D, Zhu F, Carruthers R, Ramanuam R, et al. Age-related multiple sclerosis severity score: disability ranked by age. Mult Scler. (2017) 23:1938–46. doi: 10.1177/1352458517760968
39. Teunissen CE, Petzold A, Bennett JL, Berven FS, Brundin L, Comabella M, et al. A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. Neurology. (2009) 73:1914–22. doi: 10.1212/WNL.0b013e3181c74c2c
hospitals rehabilitation study. *Mult Scler.* (2018), doi: 10.1177/1352458518795416

48. Belanger M, Magistretti PJ. The role of astroglia in neuroprotection. *Dialogues Clin Neurosci.* (2009) 11:281–95.

49. Neumann H, Kotter MR, Franklin RJ. Debris clearance by microglia: an essential link between degeneration and regeneration. *Brain.* (2009) 132 (Pt 2):288–95. doi: 10.1093/brain/aws109

50. Suarez-Calvet M, Araque Caballero MA, Kleinberger G, Bateman RJ, Fagan AM, Morris JC, et al. Early changes in CSF sTREM2 in dominantly inherited Alzheimer’s disease occur after amyloid deposition and neuronal injury. *Sci Transl Med.* (2016) 8:369ra178. doi: 10.1126/scitranslmed.aag1767

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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