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Elevated MBP in Serum Oligodendrocyte-Derived EVs Distinguishes Primary Progressive from Relapsing-Remitting Multiple Sclerosis: A Pilot Study

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Abstract

Roughly 15% of individuals with multiple sclerosis (MS) present with a progressive course from the very beginning; this subtype, primary progressive (PP) MS, remains challenging regarding both diagnosis and management and is linked to unfavorable outcomes. Brain-derived extracellular vesicles (EVs) detectable in circulation, together with their internal proteins, may serve as indicators of disease processes. We examined whether the levels of MBP and MOG within oligodendrocyte-derived EVs (ODEVs) might operate as biomarkers for MS and assist in distinguishing clinical forms of the illness. In total, 136 participants were studied (7 CIS, 18 PPMS, 49 RRMS) along with 70 matched healthy controls (HC). ODEVs were isolated from serum through immuno-capture using an anti-MOG antibody, and MBP/MOG cargoes were quantified by ELISA. MBP concentrations in ODEVs were markedly higher in CIS ($p < 0.001$), RRMS ($p < 0.001$) and PPMS ($p < 0.001$) when compared with HC and showed associations with EDSS and MSSS scores. Importantly, MBP content in PPMS was also significantly elevated relative to RRMS ($p = 0.004$) and CIS ($p = 0.03$). Logistic regression and ROC analyses supported these findings. A low-burden blood assay assessing MBP within ODEVs may represent a valuable adjunct for MS diagnostics.

Keywords: Multiple sclerosis, Primary progressive multiple sclerosis, Extracellular vesicles, Exosomes, Oligodendrocytes, MOG

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Introduction

Multiple sclerosis (MS) is a CNS-directed, immune-mediated demyelinating disorder [1] that primarily impacts young adults. Its prevalence ranges from 50–300/100,000, and approximately 2–3 million people worldwide are estimated to have MS [2]. Autoimmune attacks in MS are directed at CNS proteins. Myelin—a layered insulating membrane essential for rapid axonal conduction—is produced by oligodendrocytes that extend processes around neuronal fibers [3]. Core myelin proteins, including MBP, MOG, and PLP, constitute major autoantigenic targets implicated in MS pathology [4-6].

The clinical evolution of MS is extremely heterogeneous, spanning from mild functional disturbance to severe progressive impairment with limited therapeutic response [7]. Patients with relapsing–remitting MS (RRMS) exhibit intermittent neurologic episodes with possible residual deficits. A proportion (15–30%) of RRMS cases transition to secondary progressive MS (SPMS). Additionally, about

15% present with primary progressive MS (PPMS), characterized by steady neurologic decline from symptom onset without typical relapses or recoveries [8]. Reliable, reproducible, blood-based biomarkers could improve early stratification of MS subtypes and inform treatment planning.

Extracellular vesicles originating in the CNS can traverse the blood–brain barrier and be retrieved from peripheral blood [9]; their protein constituents may act as indicators of disease states. MOG expression—primarily found in oligodendrocytes at the RNA level (https://www.proteinatlas.org/ENSG00000204655-MOG/single+cell+type, accessed 16 September 2022)—enables the identification of ODEVs [10]. Applying a recently developed enrichment method, we purified ODEVs from the serum of individuals with CIS, RRMS, PPMS, and HC to test whether MBP and MOG levels within these vesicles could function as diagnostic or prognostic biomarkers in MS.

Results and Discussion

Cohort characteristics

Table 1 summarizes the demographic and clinical data for all participants. A total of 67 MS patients (49 RRMS, 18 PPMS) met the 2017 McDonald criteria for MS diagnosis [11], and 7 individuals were categorized as CIS. Some group-related gender differences were noted **(Table 1)**, but these were no longer present when RRMS and PPMS were analyzed together. PPMS patients demonstrated a significantly older age at onset compared with RRMS ($p < 0.01$). As anticipated, EDSS and MSSS scores were greater in PPMS than in both RRMS and CIS **(Table 1)**. Mean age across groups was similar.

Table 1. Study cohort demographic and clinical characteristics.

	CIS	RRMS	PPMS	HC
Participants (n)	7	49	18	62
Female participants (n, %)	2 (28.6) &	32 (65.3) &,¥	5 (38.5) ¥,#	32 (51.6) #
Age (years ± SD)	44.14 ± 7.10	45.80 ± 9.46	54.00 ± 12.53	51.14 ± 12.29
Duration of disease (years ± SD)	10.14 ± 6.69	14.71 ± 9.02	12.44 ± 11.98	n.a.
Age of disease onset (years ± SD)	34.00 ± 3.37	31.08 ± 9.37 *	41.56 ± 12.26 *	n.a.
EDSS score (mean ± SD)	1.57 ± 1.51 $	2.62 ± 1.94 £	5.08 ± 1.40 $,£	n.a.
MSSS score (mean ± SD)	1.38 ± 1.25 °	2.72 ± 2.32 §	6.97 ± 2.06 °,§	n.a.

&p = 0.01; ¥ p < 0.01; # p = 0.04; * p < 0.01; $ p < 0.001; £ p < 0.001; ° p < 0.001; § p < 0.001.

*HLA-DRB1*15 genotyping*

All participants were screened for the HLA-DRB1*15 variant, considering both single- and double-allele carriers. This allele appeared far more frequently among individuals with MS (CIS + RRMS + PPMS) than in healthy controls, with a highly significant association ($p = 2.8 \times 10^{-5}$, OR = 10.58, 95% CI: 2.82–67.99), reaffirming its strong genetic link to MS.

ODEVs characterization

The isolated ODEVs were examined following the MISEV criteria established by the International Society for Extracellular Vesicles [12]. An example of the Exo-Check Antibody Array outcome is provided in **Figure 1a**, showing detection of exosome-linked proteins (CD63, CD81, ALIX, FLOT1, ICAM1, EpCam, ANXA5, TSG101) together with positive assay controls. The Golgi marker GM130 was absent, indicating no appreciable cellular contamination.

Morphological analysis via immunogold TEM revealed variably sized vesicles, and anti-OMG labeling confirmed the selective enrichment of ODEVs through visible dark immunogold deposits **(Figure 1b)**.

For quantification, five samples each from HC, PPMS, and RRMS were evaluated. The mean particle counts did not differ significantly (HC: 6.3×10^{10}/mL, SD 2.6×10^{10}; PPMS: 6.0×10^{10}/mL, SD 2.2×10^{9}; RRMS: 1.1×10^{11}/mL, SD 1.2×10^{10}) with ANOVA $p = 0.10$ **(Figure 1c)**. Vesicle diameters were also comparable across conditions (HC: 74.2 ± 12.3 nm; PPMS: 88.9 ± 3.2 nm; RRMS: 82.5 ± 8.5 nm) with $p = 0.10$.

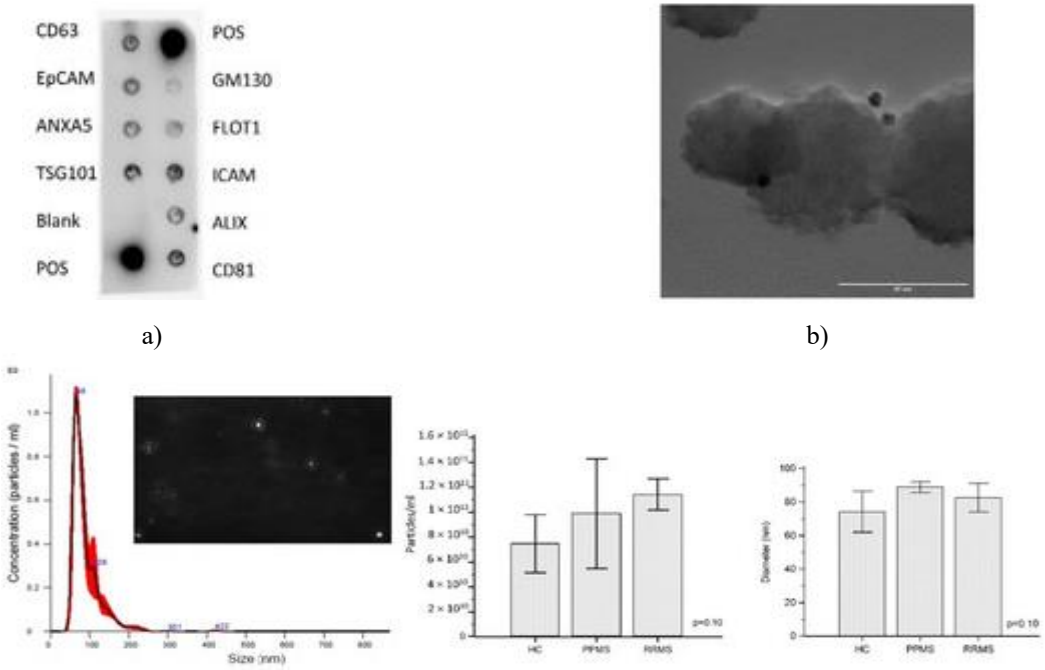


Figure 1. ODEV characterization.
(a) Exo-Check™ membrane showing vesicle-related proteins and control spots (positive controls, blank, and GM130).
(b) Immunogold TEM illustrating OMGp-positive vesicles; scale = 100 nm.
(c) NTA-based size and concentration profile from an RRMS sample, with summary values (± SD) from five samples per group and ANOVA statistics.

MBP concentration in enriched ODEVs is increased in MS patients

Using sandwich ELISA, levels of MBP and MOG within ODEVs from HC, CIS, RRMS, and PPMS were measured. Distribution tests (Kolmogorov–Smirnov) indicated non-normality; therefore, a Kruskal–Wallis analysis was applied. A strong overall difference in MBP levels was detected ($p < 0.0001$) **(Figure 2a)**.

Pairwise comparisons (Dwass–Steel–Critchlow–Fligner) showed that HC samples had distinctly lower MBP values (median 17.85 pg/mL, IQR 14.00–24.39) relative to CIS (78.73 pg/mL, IQR 65.78–91.20, $p < 0.001$), RRMS (83.76 pg/mL, IQR 71.05–95.03, $p < 0.001$) and PPMS (136.44 pg/mL, IQR 86.32–225.67, $p < 0.001$).

PPMS patients displayed significantly higher MBP concentrations compared with RRMS ($p = 0.004$) and CIS ($p = 0.03$). CIS and RRMS themselves did not differ.

Combining CIS and RRMS (RRMS + CIS) produced MBP levels (median 82.16 pg/mL, IQR 68.09–93.51) markedly below those of PPMS (136.44 pg/mL, IQR 86.32–225.67, $p < 0.01$) **(Figure 2b)**.

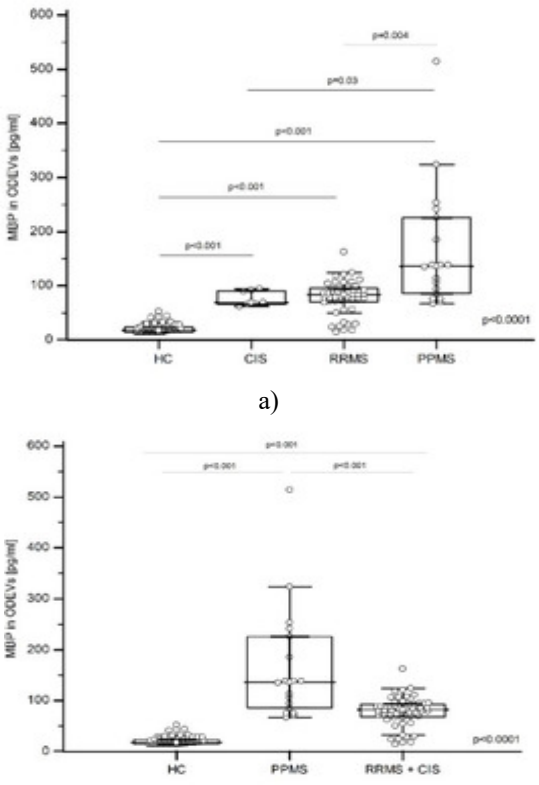


Figure 2. MBP in enriched ODEVs.
(a) Comparison of HC, CIS, RRMS, and PPMS MBP values with medians/IQRs, Kruskal–Wallis global test, and post-hoc statistics.

(b) MBP comparisons across HC, combined CIS + RRMS, and PPMS with corresponding medians and IQRs and non-parametric test results.

A logistic regression analysis was performed next, using disease category (CIS, RRMS, PPMS) or HC status as the dependent variable. Independent variables included MBP levels in ODEVs, age, sex, and presence of at least one HLA-DRB1*15 allele. The analysis covered all 136 participants. The results demonstrated that MBP concentration in ODEVs was the only factor that significantly differentiated MS cases from controls (χ^2 = 113.61; $p < 0.0001$), while none of the other covariates reached significance. The classification accuracy of this model was 90.83%.

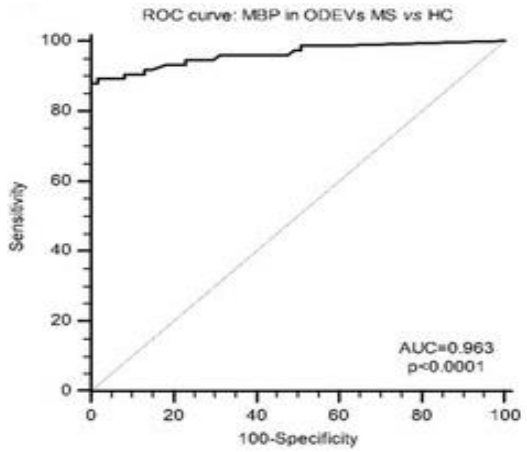
A second model was applied with the dependent outcome set as RRMS + CIS versus PPMS. Again using the same covariates, MBP levels remained a significant predictor ($p = 0.003$) together with sex ($p = 0.05$) and age ($p = 0.004$) (overall model fit: $\chi^2 = 40.91$; $p < 0.0001$).

In contrast, although MOG was measurable in ODEVs from MS and HC groups, no meaningful differences emerged across categories (data not displayed).

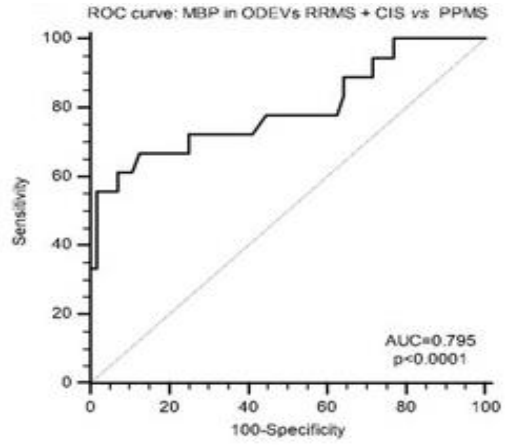
ROC curve analysis

Receiver operating characteristic analysis was then conducted to determine the diagnostic value of MBP in ODEVs. Comparing HC with MS groups combined (CIS + RRMS + PPMS) yielded an AUC of 0.963 (95% CI: 0.916–0.988), accompanied by 97.84% sensitivity and 100.00% specificity ($p < 0.0001$) **(Figure 3a)**.

When contrasting PPMS with RRMS + CIS, the ROC curve showed an AUC of 0.795 (95% CI: 0.685–0.880), with 66.67% sensitivity and 87.50% specificity ($p < 0.0001$) **(Figure 3b)**.



a)

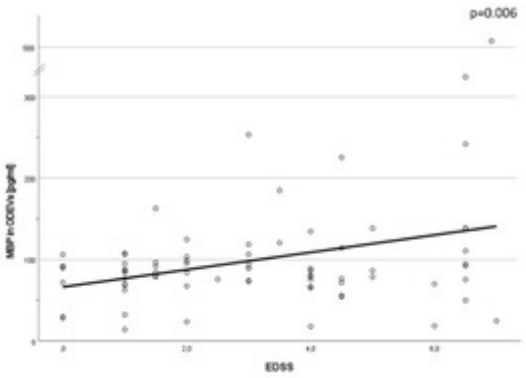


b)

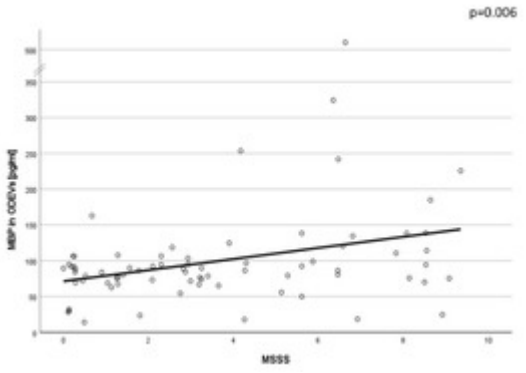
Figure 3. (a) ROC curve for MBP levels separating HC from combined MS groups; AUC and p are indicated. (b) ROC curve distinguishing PPMS from CIS + RRMS; AUC and significance are shown.

Correlation with clinical scores

Pearson analyses revealed a positive association between MBP concentrations in ODEVs and EDSS ($p < 0.01$, $r = 0.32$, 95% CI: 0.09–0.51) as well as MSSS ($p < 0.01$, $r = 0.32$, 95% CI: 0.09–0.51) **(Figures 4a and 4b)**.



a)



b)

Figure 4. (a) Pearson correlation: MBP in ODEVs vs. EDSS.
(b) Pearson correlation: MBP in ODEVs vs. MSSS.

This case–control pilot investigation demonstrates that quantifying MBP transported within serum-derived

oligodendrocyte extracellular vesicles provides a highly sensitive and specific method for distinguishing MS patients from healthy individuals. More notably, the MBP load in these vesicles also allows separation of PPMS from RRMS cases. Furthermore, MBP levels in ODEVs positively relate to disease burden as measured by EDSS and MSSS.

MS exhibits a wide degree of clinical variability, ranging from mild presentations to progressively disabling forms [7]. Among these, PPMS typically carries a poorer prognosis, and many disease-modifying therapies that benefit RRMS provide limited advantage in PPMS, though some benefit has been observed for rituximab [13] and ocrelizumab [14]. Early stratification into clinical phenotypes is therefore essential for tailored management. Current MS diagnosis relies on a mixture of clinical evaluation, MRI findings, and laboratory indicators [11], but accessible blood-based markers capable of subtype discrimination are still lacking. MBP quantification in ODEVs may address this gap.

MBP represents roughly 30% of myelin proteins [15] and has long been known as a principal autoimmune target in MS. Earlier observations suggested limited usefulness of CSF MBP for diagnosis [16], although later findings proposed a role in tracking disability progression, particularly in SPMS [17]. Oligodendrocyte-derived exosomes carry abundant myelin proteins [18], and CNS-derived vesicles can traverse the BBB [9]. Galazka *et al*. identified MOG and other myelin components in total serum EVs from MS patients, especially during relapses, but did not observe MBP differences among disease groups [19]. Differences between their report and our findings are likely due to the use of enriched ODEVs rather than total EV isolates, and the inclusion of PPMS in our cohort, which their work did not examine. Their study also suggested that circulating vesicles loaded with myelin antigens might sustain or amplify autoimmune activation [19]. Our data support the broader concept that CNS-derived proteins within ODEVs can be quantified and hold diagnostic relevance.

An additional observation is that CIS and RRMS show comparable MBP concentrations. Considering that CIS does not always progress to RRMS, future longitudinal studies may clarify whether ODEV-MBP can identify CIS patients at higher risk for conversion.

Study limitations include the relatively small sample group and the absence of pharmacological-treatment stratification. Larger studies, ideally involving early-stage patients, will be necessary to test whether MBP levels in ODEVs can reliably forecast MS phenotype development.

Materials and Methods

Study cohort

This case-control investigation recruited 136 participants. Among them were 67 individuals diagnosed with MS and 7 with CIS, all enrolled at the Multiple Sclerosis Unit of the IRCCS Fondazione Don Carlo Gnocchi in Milan. MS diagnoses followed the revised McDonald criteria [11], assigning 18 PPMS patients and 49 RRMS patients (all in remission when blood samples were collected). Demographic and clinical data, including age, disease onset, disease duration, EDSS [20], treatment regimens, and MSSS (calculated following Roxburgh [21]), were recorded in a pseudo-anonymized dataset.

A control group of 70 neurologically healthy adults was recruited from hospital staff. Exclusion criteria for HC included any autoimmune or neurological illnesses and age < 35 years, minimizing the possibility of including individuals in early preclinical stages of MS. Written informed consent was collected from every participant. The study adhered to the Declaration of Helsinki and received approval from the Don Carlo Gnocchi ONLUS Foundation ethics committee (Protocol #11_27/06/2019). **Table 1** summarizes demographic and clinical features.

Collection of serum

Peripheral blood (25 mL) was drawn into SST II Advance tubes (BD Vacutainer®) following standardized protocols. The samples were left at room temperature to clot for 10–20 minutes, after which centrifugation was performed at 1500 × g for 10 minutes. The serum was then carefully separated, portioned into aliquots, and stored at −80 °C for subsequent experiments.

Enrichment of ODEVs

To isolate ODEVs, 250 µL of serum was processed according to a modified two-step protocol [22]. Initially, 150 µL of calcium- and magnesium-free PBS and 15 µL of Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific, Waltham, MA, USA) were added, followed by centrifugation at 3000 × g for 30 minutes at 4 °C. The supernatant was then combined with 126 µL of ExoQuick® exosome precipitation solution (System Bioscience, Palo Alto, CA, USA) and centrifuged at 1500 × g for 30 minutes at 4 °C.

The pellet was resuspended in 350 µL PBS, to which 50 µL of 3% BSA and 2 µg biotinylated anti-MOG antibody (Bioss Antibodies, Boston, MA, USA) were added. After 1 hour of incubation at room temperature, 10 µL of Pierce™ Streptavidin UltraLink™ Resin and 40 µL of 3% BSA were introduced and incubated for 30 minutes at room temperature. Following centrifugation at 800 × g for 10 minutes at 4 °C, pellets were resuspended in 100 µL 0.05 M glycine-HCl (pH 3.0) and vortexed for 10 seconds. After another centrifugation step at 4000 × g for 10 minutes at 4 °C, pellets were supplemented with 25 µL

10% BSA, 10 µL Tris-HCl, and 15 µL Halt™ protease/phosphatase inhibitors.

From each preparation, 20 µL of intact ODEVs were frozen at −80 °C, and the remainder was lysed with 365 µL M-PER™ and 15 µL protease/phosphatase inhibitors, subjected to two freeze-thaw cycles, and stored at −80 °C.

Characterization of ODEVs

Enriched ODEV lysates were analyzed for standard exosomal markers using a Western blot-based array (Exo-Check, System Bioscience), as per manufacturer instructions. The array contains 12 spots: eight for exosomal proteins (CD63, CD81, ALIX, FLOT1, ICAM1, EpCam, ANXA5, TSG101) and four controls (two positives, one background, and GM130 for monitoring cellular contamination). Membranes were visualized with Clarity Max ECL substrate and imaged using a ChemiDoc™ Gel Imaging System (Bio-Rad).

For TEM, 5 µL of ODEVs were adsorbed on 200-mesh formvar-carbon grids for 10 minutes, then blotted. Grids were washed twice in 50 µL 0.1% BSA in PBS, incubated in a humid chamber with 50 µL primary antibody (anti-OMG, 1:100) for 3 hours, washed, and then incubated for 1 hour with 30 µL 1:50 anti-IgG-gold conjugate (10 nm). Five washes with buffer and five with water were performed before negative staining with 1% Uranyl Acetate for 10 seconds. Imaging was performed on a JEOL JEM 2100Plus TEM at 200 kV with an 8-megapixel Gatan Rio CMOS camera.

Particle size and concentration were determined via NTA for five samples per group (HC, PPMS, RRMS) using a NanoSight NS300 equipped with a 488 nm laser, flow-cell top plate, and syringe pump. Samples were diluted 1:250 in PBS. Three 60-second videos were recorded per sample, and mean sizes were calculated using NTA v3.4 (detection threshold = 5).

Immunoassays

MBP and MOG levels in undiluted ODEV lysates were quantified using commercial sandwich ELISA kits (MyBiosource, cat #MBS2502574 and MBS928110), following manufacturer instructions. All samples and standards were measured in duplicate.

*Genotyping for HLA-DRB1*15*

HLA-DRB1*15 alleles were identified via SNP rs3135388 using TaqMan™ allelic discrimination (probe C__27464665_30, Thermo Fisher). PCR conditions included 95 °C hot start for 10 min, then 40 cycles of 94 °C for 15 s and 60 °C for 1 min with fluorescence acquisition at 60 °C. Reactions (10 µL) contained 1 µL DNA (50 ng/µL), TaqMan Genotyping Master Mix, and controls for all genotypes and a negative control, run on a CFX96 instrument (Bio-Rad) in 96-well plates.

Statistical analyses

Group differences in demographic and clinical features among HC, CIS, RRMS, and PPMS were evaluated using the Chi-square or Student's t-test. MBP and MOG data were non-normally distributed (Kolmogorov–Smirnov test), prompting the use of Mann–Whitney U or Kruskal–Wallis tests. Pairwise comparisons employed Dwass–Steel–Critchlow–Fligner post hoc tests. NTA results (particle size and concentration) were compared via ANOVA.

ROC curves were constructed to evaluate the diagnostic potential of ODEV biomarkers, calculating AUC, sensitivity, specificity, and 95% CI. AUC interpretation: 0.7–0.8 = acceptable, 0.8–0.9 = excellent, >0.9 = outstanding [23]. Logistic regressions considered disease status as the dependent variable, with ODEVs MBP, age, sex, and HLA-DRB1*15 as covariates. Correlations between biomarkers and clinical parameters were assessed using Pearson's correlation. Significance was defined as $p \leq 0.05$. Software used included MedCalc 14.10.2, SPSS v27, jamovi 2.2, and OpenEpi (accessed 6 October 2022).

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Conflict of interest: None

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Ethics statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Ethics Committee of IRCCS Fondazione Don Carlo Gnocchi (Protocol number: #11_27/06/2019).

Written informed consent was obtained from all subjects involved in the study.

References

1. Thompson AJ, Baranzini S, Geurts J, Hemmer B, Ciccarelli O. Multiple sclerosis. Lancet. 2018;391(10130):1622–36.
2. Browne P, Chandraratna D, Angood C, Tremlett H, Baker C, Taylor BV, et al. Atlas of Multiple Sclerosis 2013: A growing global problem with widespread inequity. Neurology. 2014;83(11):1022–4.
3. Dubois-Dalcq M, Armstrong R. The cellular and molecular events of central nervous system remyelination. Bioessays. 1990;12(9):569–76.
4. Krogsgaard M, Wucherpfennig KW, Cannella B, Hansen BE, Svejgaard A, Pyrdol J, et al.

Visualization of myelin basic protein (MBP) T cell epitopes in multiple sclerosis lesions using a monoclonal antibody specific for HLA-DR2-MBP 85-99 complex. J Exp Med. 2000;191(8):1395–412.
5. Kerlero de Rosbo N, Milo R, Lees MB, Burger D, Bernard CC, Ben-Nun A. Reactivity to myelin antigens in multiple sclerosis. Peripheral blood lymphocytes respond predominantly to myelin oligodendrocyte glycoprotein. J Clin Invest. 1993;92(5):2602–8.
6. Correale J, McMillan M, McCarthy K, Le T, Weiner LP. Isolation and characterization of autoreactive proteolipid protein-peptide specific T-cell clones from multiple sclerosis patients. Neurology. 1995;45(7):1370–8.
7. Filippi M, Bar-Or A, Piehl F, Preziosa P, Solari A, Vukusic S, et al. Multiple sclerosis. Nat Rev Dis Primers. 2018;4(1):43.
8. Miller DH, Leary SM. Primary-progressive multiple sclerosis. Lancet Neurol. 2007;6(10):903–12.
9. Shi M, Liu C, Cook TJ, Bullock KM, Zhao Y, Ginghina C, et al. Plasma exosomal α-synuclein is likely CNS-derived and increased in Parkinson's disease. Acta Neuropathol. 2014;128(5):639–50.
10. Ohmichi T, Mitsuhashi M, Tatebe H, Kasai T, Ali El-Agnaf OM, Tokuda T. Quantification of brain-derived extracellular vesicles in plasma as a biomarker to diagnose Parkinson's and related diseases. Park Relat Disord. 2019;61:82–7.
11. 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. 2018;17(2):162–73.
12. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the International Society for Extracellular Vesicles. J Extracell Vesicles. 2018;7(1):1535750.
13. Hawker K, O'Connor P, Freedman MS, Calabresi PA, Antel J, Simon J, et al. Rituximab in patients with primary progressive multiple sclerosis: Results of a randomized double-blind placebo-controlled multicenter trial. Ann Neurol. 2009;66(4):460–71.
14. Montalban X, Hauser SL, Kappos L, Arnold DL, Bar-Or A, Comi G, et al. Ocrelizumab versus placebo in primary progressive multiple sclerosis. N Engl J Med. 2017;376(3):209–20.
15. Einstein ER, Robertson DM, Dicaprio JM, Moore W. The isolation from bovine spinal cord of a homogeneous protein with encephalitogenic activity. J Neurochem. 1962;9(6):353–61.
16. Greene DN, Schmidt RL, Wilson AR, Freedman MS, Grenache DG. Cerebrospinal fluid myelin basic protein is frequently ordered but has little value: A test utilization study. Am J Clin Pathol. 2012;138(2):262–72.
17. Herman S, Khoonsari PE, Tolf A, Steinmetz J, Zetterberg H, Åkerfeldt T, et al. Integration of MRI and protein and metabolite CSF measurements to enable early diagnosis of secondary progressive multiple sclerosis. Theranostics. 2018;8(17):4477–90.
18. Fabriek BO, Zwemmer JN, Teunissen CE, Dijkstra CD, Polman CH, Laman JD, Castelijns JA. In vivo detection of myelin proteins in cervical lymph nodes of MS patients using ultrasound-guided fine-needle aspiration cytology. J Neuroimmunol. 2005;161(1–2):190–4.
19. Galazka G, Mycko MP, Selmaj I, Raine CS, Selmaj KW. Multiple sclerosis: Serum-derived exosomes express myelin proteins. Mult Scler. 2018;24(4):449–58.
20. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). Neurology. 1983;33(11):1444–52.
21. Roxburgh RH, Seaman SR, Masterman T, Hensiek AE, Sawcer SJ, Vukusic S, et al. Multiple Sclerosis Severity Score: Using disability and disease duration to rate disease severity. Neurology. 2005;64(7):1144–51.
22. Goetzl EJ, Schwartz JB, Abner EL, Jicha GA, Kapogiannis D. High complement levels in astrocyte-derived exosomes of Alzheimer disease. Ann Neurol. 2018;83(4):544–52.
23. Mandrekar JN. Receiver operating characteristic curve in diagnostic test assessment. J Thorac Oncol. 2010;5(9):1315–6.