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Oligoclonal IgM band patterns in multiple sclerosis: A two-center study

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ABSTRACT

Background: Cerebrospinal fluid (CSF) oligoclonal IgM bands (OCMBs) have been suggested as prognostic biomarkers in MS, but serum OCMBs meaning is still uncertain.
Objectives: We aimed to assess frequency and clinical relevance of all OCMB patterns.
Methods: In this retrospective cohort study, 136 paired sera-CSF from consecutive persons with MS (pwMS) were tested in 2 centers for OCMBs using isoelectric focusing-immunoblotting. Active disease was defined as clinical or radiological relapse occurring during two-year follow-up. Predictors of active disease were analyzed with logistic regressions and Kaplan-Meier survival curves.
Results: OCMBs were found in 6.6 % of pwMS as unique-to-CSF (pattern #2), and in 20.6 % as identical in serum-CSF (pattern #4), without between-cohort difference. Active disease was more frequent in those with pattern #2 (88.9 %) and #4 (64.3 %) than in those OCMB-negative (33.3 %, $p < 0.001$). In multivariate analysis, pattern #2 (OR: 15.9; 95 % CI [1.8–136]), and pattern #4 (OR: 3.3 95 % CI [1.3–8.3]) were independent predictors of active disease. In survival analysis, pattern #2 ($p < 0.001$) and #4 ($p = 0.017$) predicted radiological relapses.
Conclusions: Our data confirm that CSF OCMB marks poor prognosis in MS. However, both OCMB pattern #4 and pattern #2, with different strength prediction, might be useful to stratify pwMS deserving more aggressive treatments, although the stratification could be achieved in the near future with more standardized and easily measurable biomarkers (e.g., serum neurofilaments).

1. Introduction

The detection of unique-to-CSF oligoclonal IgG bands (OCBs) allows to define the dissemination in time in persons with clinically isolated syndrome (CIS), and to diagnose MS early (Thompson et al., 2018). After diagnosis, the trajectory of the disease remains mostly unpredictable, despite the many prognostic biomarkers identified so far, mainly due to their lack of validation and practical implementation in clinical settings (Rotstein and Montalban, 2019). Among them, unique-to-CSF oligoclonal IgM bands (OCMBs) have been associated to a detrimental disease course in several studies (Villar et al., 2003; Espino et al., 2015; Oechtering et al., 2021; Thangarajh et al., 2008; Villar et al., 2002a), with good interlaboratory reproducibility of a standardized isoelectric focusing (IEF)-immunoblotting procedure (Espino et al., 2015), suggesting that they could represent a promising prognostic marker (Teunissen et al., 2015). However, the lack of association between MS prognosis and OCMBs has also been reported (Frau et al., 2018; Schneider et al., 2007).

Most studies on persons with MS (pwMS) focused mainly on unique-to-CSF OCMBs, which correspond to pattern #2, in accordance with the rules established for IgG OCBs (Andersson et al., 1994). In pwMS, the overall frequency of intrathecal IgM synthesis, assessed with OCMB

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pattern #2 (qualitatively, and with the highest analytical sensitivity), or the Reiber formulas (quantitatively), is 40.1 % (Fonderico et al., 2021), with a wide range of between-study variability: 23.2 % (Pfuhl et al., 2019), 26.0 % (Oechtering et al., 2021), 29.6 % (Thangarajh et al., 2008), 46.1 % (Villar et al., 2002b), 68.7 % (Perini et al., 2006). Accordingly, inter-study variability concerns the association between OCMBs and more active inflammatory MS phenotypes (Oechtering et al., 2021; Villar et al., 2002a; Perini et al., 2006; Villar et al., 2005), or worse long-term outcomes (Villar et al., 2003; Thangarajh et al., 2008; Capuano et al., 2021). Conversely, only a few studies indirectly investigated the frequency and clinical relevance of other OCMB patterns, particularly that characterized by oligoclonal IgM produced peripherally, and therefore detectable on both serum and CSF (pattern #4) (Espino et al., 2015; Capuano et al., 2021; Villar et al., 2001).

In our study, using key clinical parameters, such as EDSS, relapse rate, and brain and spinal cord MRI activity, we aimed to assess both the frequency of all OCMB patterns and their prognostic meaning in RRMS.

2. Materials and methods

2.1. Participants and inclusion criteria

In this retrospective cohort study, pwMS consecutively admitted at the IRCCS Policlinico San Martino of Genoa, and at the IRCCS Fondazione Mondino of Pavia were enrolled. The project was approved by the Institutional Review Board of the IRCCS Policlinico San Matteo, Pavia (project's code, 0020308/23). All participants provided written informed consent according to the Declaration of Helsinki (World Medical A, 2013). Inclusion criteria were the following: diagnosis of relapsing-remitting MS (Thompson et al., 2018); availability of frozen paired serum and CSF samples, and of routine CSF analysis results; availability of brain and spinal cord MRI data; at least one clinical and/or radiological follow-up findings in the following two years from CSF sampling. Lumbar puncture was performed prior to any acute-phase treatment, in line with good clinical practice, as well as follow-up clinical assessment and MRI scans. Active disease within two years from diagnosis was defined as follows: at least one clinical relapse, one radiological new/enlarging lesion, or both. Type of, and number of pwMS on high-efficacy therapies (HETs) were as follows: natalizumab (n = 30), fingolimod (n = 6), ozanimod (n = 3), siponimod (n = 2), cladribine (n = 9), ofatumumab (n = 5), ocrelizumab (n = 30).

Fifty-four controls were patients with other inflammatory neurological diseases (OIND; n = 39), including autoimmune encephalitis (n = 14), non-MS CNS autoimmune demyelinating syndromes (n = 19), and patients with non-inflammatory neurological diseases (NIND; n = 15). Paired serum and CSF samples underwent routine analysis upon arrival in both the centers (Gastaldi et al., 2017). Aliquots of the samples were stored in repositories at −20 °C until analysis.

2.2. Oligoclonal IgM band detection

For OCMB detection, both the laboratories used a shared protocol of IEF-immunoblotting method previously validated for the application in daily clinical practice (Espino et al., 2015; Villar et al., 2001), with slight modifications. Briefly, serum samples were 1:800 diluted in saline solution to equalize IgM concentrations in paired serum and CSF samples, in accordance with the physiological serum to CSF gradient for IgM, and assuming an intact blood-CSF barrier. Samples were incubated with 50 mmol dithiothreitol at pH 9.5 for 30 min to reduce IgM. IEF was performed on agarose gel [25 ml of distilled water containing sorbitol 3.6 g, agarose IEF (Amersham-Pharmacia) 0.3 g, and ampholytes 2.5 ml of Pharmalyte pH 5–8 (Amersham-Pharmacia)], and run at 5 W for 30 min, and then at 10 W until focusing was completed, after 1500 V/h. Proteins were transferred to a nitrocellulose membrane, and stained with an alkaline phosphatase-conjugated goat anti-human IgM antibody (Jackson Scientific), with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium, or 3,3′-diaminobenzidine as substrates. Interpretation of immunoblotting results followed consensus-based criteria (Andersson et al., 1994). Fig. 1 shows typical examples of the two main oligoclonal patterns, as detected in the two laboratories of Genoa and Pavia, namely pattern #2 and #4 (Fig. 1). Notably, bands in MS samples tended to be more frequently less focused and less sharp than those in samples from patients with non-MS inflammatory autoimmune disorders of the CNS.

2.3. Statistical analysis

Mean and standard deviation were used for continuous parametric variables, while median, percentage, and interquartile range for dichotomous and non-parametric variables. Cohort differences in clinical and demographic characteristics were investigated with Pearson Chi-Square test for dichotomous variables, Wilcoxon signed Rank-test for continuous non-parametric variables, and Independent *T-Test* for continuous parametric variables. Associations between OCMB patterns and active disease were analyzed with Pearson Chi-square test and Fischer's exact test. Logistic regression with age, sex, EDSS score at sampling, high-efficacy therapy as initial treatment, and disease duration as covariates was run in the whole cohort of pwMS. Survival curves were calculated with Kaplan-Meier analysis. Probability values <0.05 were considered significant. All statistical analyses were performed with the SPSS (IBM) program.

3. Results

3.1. Cohort characteristics

Among the 136 pwMS enrolled, 90 were female (66 %), and mean age was 35 years (±10.3). After two years of follow-up, 77 had stable disease, and 59 had active disease (Table 1).

Between active vs stable pwMS, no significant difference in age and EDSS score was found. PwMS with active disease showed significantly higher proportion of male sex (42 % vs 27 %, p = 0.048), disease duration at sampling (0 (0–1) vs 0 (0–0), p = 0.003), and high efficacy therapy as initial treatment (71.2 % vs 55.8 %, p = 0.049).

3.2. Frequency of OCMB patterns

Overall, 37/136 (27.2 %) of pwMS had OCMBs. Pattern #4 was detected in 28 of them (20.6 %), whereas pattern #2 in only 9 (6.6 %) (Table 1). None of the pwMS had pattern #3, or pattern #5. The frequency of the patterns did not substantially differ between the two

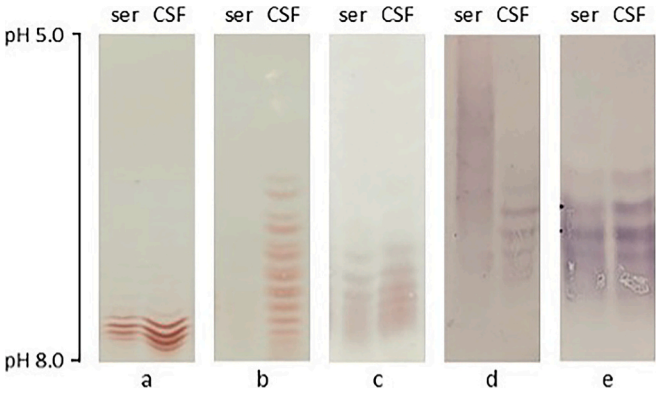

Fig. 1. Immunoblots of oligoclonal IgM bands (OCMBs) of paired serum (ser) and cerebrospinal fluid (CSF) samples from a patient with monoclonal IgM gammopathy (a; positive control), patients with autoimmune encephalitis (b, d; unique-to-CSF OCMBs, pattern #2), persons with MS (c, e; identical serum and CSF OCMBs, pattern #4); run performed at Genoa (a-c), and Pavia (d, e) laboratories.

Table 1
Demographic and clinical features of persons with MS with active vs stable disease in the whole cohort.

	All Patients (n = 136)	Active Disease (n = 59)	Stable Disease (n = 77)	p-value†	Univariate (OR [CI95 %])	p-value§	Multivariate (OR [CI95 %])	p-value‡
Age, years, mean ± SD	35 ± 10.3	36 ± 11.3	34 ± 9.4	0.079	1.02 [0.9–1.05]	0.18	1.05 [0.9–1.05]	0.4
Female Sex, n (%)	90 (66)	34 2(58)	56 (73)	**0.048**	1.9 [0.9–4.0]	0.07	1.5 [0.7–3.5]	0.3
EDSS score, median (IQR)	2.0 (1.0–2.5)	2.0 (1.5–2.5)	2.0 (1.0–2.25)	0.338	1.1 [0.8–1.6]	0.48	1.0 [0.6–1.5]	0.9
Disease duration at sampling, months, median (IQR)	0 (0–0)	0 (0–1)	0 (0–0)	**0.003**	1.1 [0.9–1.2]	0.07	1.1 [1.0–1.2]	**0.049**
HET initial treatment (%)	62.5	71.2	55.8	**0.049**	1.9 [0.9–4]	0.07	1.6 [0.7–3.8]	0.2
OCMB Pattern #2, n (%)	9 (6.6 %)	8 (13.5 %)	1 (1.3 %)	**0.005**	11.9 [1.5–98)]	**0.021**	15.9 [1.8–136]	**0.012**
OCMB Pattern #4, n (%)	28 (20.6 %)	18 (30.5 %)	10 (12.9 %)	**0.011**	2.9 [1.3–6.9]	**0.015**	3.3 [1.3–8.3]	**0.010**

OCMBs, oligoclonal IgM bands; EDSS, expanded disability status scale; HET, high-efficacy therapy; pattern #2, unique-to-CSF OCMBs; pattern #4, identical serum and CSF OCMBs; Active vs Stable disease differences analyzed with Pearson Chi-square/*t*-Test/Mann-Whitney *U* Test; OR = Odds Ratio; statistical differences between active and stable disease (p-value†); significance of predictors in univariate (p-value§), and multivariate (p-value‡) binary logistic regressions for active disease prediction.

centers (Table 1s, supplementary material). As for the controls, the frequencies of OCMB detection in persons with OIND were slightly higher for pattern #2 (10.2 %), and slightly lower for pattern #4 (15,1 %) than those found in pwMS (Table 3).

3.3. Demographic and clinical differences across groups

Table 2 shows demographic and clinical features of OCMB-negative, OCMB pattern #2 and OCMB pattern #4 pwMS. Female sex prevalence was significantly higher in the OCMB-negative group, while no difference in age, disease duration and EDSS was found. The frequency of unique-to-CSF oligoclonal IgG bands was not different across groups, both in the whole cohort (Table 2) and in single-center cohorts (Table 2s, supplementary material).

Table 2
Demographic and clinical features of OCMB pattern#2-positive, OCMB pattern#4-positive, and OCMB-negative persons with MS in the whole cohort.

	All patients (n = 136)	OCMB Pattern #2 (n = 9)	OCMB Pattern #4 (n = 28)	OCMB-negative (n = 99)	p-value
Age, years, mean (± SD)	35 ± 10.3	33 ± 9.6	35 ± 10.9	35 ± 10.4	0.84
Female Sex, n (%)	90 (66)	4 (44)	14 (50)	72 (73)	**0.029**
EDSS, median (IQR)	2.0 (1.0–2.5)	1.5 (1.0–2.0)	2.0 (1.5–2.0)	2.0 (1.0–2.5)	0.45
Follow-up duration, months, median (IQR)	19 (13–41)	16 (10–37)	22 (15–40)	19 (12–43)	0.47
Disease duration at sampling, months, median (IQR)	0 (0–0)	0 (0–0.5)	0 (0–0)	0 (0–0)	0.98
HET initial treatment, n (%)	85 (62.5)	7 (78)	21 (75)	57 (58)	0.15
Active disease, n (%)	59 (43.4)	8 (88.9)	18 (64.3)	33 (33.3)	**<0.001**
OCBs, n (%)	109 (80.1)	7 (77.7)	25 (89.3)	77 (77.7)	0.6

OCMBs, oligoclonal IgM bands; pattern #2, unique-to-CSF OCMBs; pattern #4, identical serum and CSF OCMBs; OCBs, unique-to-CSF oligoclonal IgG bands; EDSS, expanded disability status scale; HET, high-efficacy therapy; differences analyzed through Pearson Chi-square/t-Test/Mann-Whitney U Test.

Table 3
Oligoclonal IgM bands in patients with other inflammatory neurological diseases (OINDs) and patients with non-inflammatory neurological diseases (NIND).

	Pattern #2 (n°/total)	Pattern #4 (n°/total)
Autoimmune encephalitis	2/14	3/14
NMDAR	1/5	1/5
LGI1	1/6	1/6
CASPR2	0/2	0/2
GABA$_A$R	0/1	1/1
CNS-ADS	0/19	2/19
NMOSD	0/10	1/10
MOGAD	0/9	1/9
CIDP	0/6	1/6
OIND	**4/39**	**6/39**
Alzheimer's disease	0/6	0/6
IIH	0/5	0/5
Chronic cerebral vasculopathy	0/2	0/2
MMN	0/1	1/1
Primary CNS lymphoma	1/1	0/1
NIND	**1/15**	**1/15**

OCMBs, oligoclonal IgM bands; pattern #2, unique-to-CSF OCMBs; pattern #4, identical serum and CSF OCMBs; ADS, CNS autoimmune demyelinating syndromes; NMOSD; neuromyelitis optica spectrum disorders; MOGAD, myelin oligodendrocyte glycoprotein antibody-associated disease; MMN, multifocal motor neuropathy; IIH, idiopathic intracranial hypertension.

3.4. Active disease in OCMB patterns

Among pwMS, both pattern #2 and pattern #4 were more frequent in those with active disease than in those with stable disease (respectively, 8/59, 13.5 % vs 1/77, 1.3 %; $p = 0.005$, and 18/59, 30.5 % vs 10/77, 12.9 %; $p = 0.011$) (Table 1).

To test the prognostic relevance of OCMBs, we performed univariate binary logistic regressions, which showed that the presence of pattern #2 and pattern #4 were single predictors of active disease (respectively, OR = 11.9 (95 %CI 1.5–98); $p = 0.015$, and OR = 2.9 (98 %CI 1.3–6.9); $p = 0.02$).

Such results were confirmed in a multivariate logistic regression model including pattern #4 and pattern #2, sex, age, EDSS, high-efficacy therapy as initial treatment, and disease duration. The model was statistically significant (χ (Rotstein and Montalban, 2019) (7) = 27.7 $p < 0.001$), with R square at 0.248, and showed that disease duration at sampling (OR = 1.1 (95 %CI 1.0–1.2) $p = 0.049$), and both pattern #2 and pattern #4 were independent predictors (respectively, OR = 15.9 (95 %CI 1.8–136); $p = 0.012$; OR = 3.3 (95 %CI 1.3–8.5); $p = 0.010$) (Table 1).

3.5. Kaplan-Meier survival analysis on radiological relapses

We finally analyzed the effect of OCMB patterns on radiological relapses with Kaplan-Meier survival analysis (Fig. 2). The probability of no radiological relapses over time in pwMS without OCMBs was significantly higher than in those carrying pattern #4 ($p = 0.017$), and even higher compared to those with pattern #2 ($p = 0.002$).

4. Discussion

In this two-center study, we confirmed that unique-to-CSF OCMBs (pattern #2) are a useful prognostic biomarker in MS, being able to predict active course at onset of the disease, and the occurrence of radiological relapses over the following two years. As a novelty, we highlighted a similar role for identical OCMBs in serum and CSF (pattern #4), which have likely been underestimated in pwMS so far.

From a biological point of view, the difference between pattern #2 and pattern #4 is substantial, as the former indicates the intrathecal production of oligoclonal IgM, whereas the latter a production that develops in periphery, with passive transfer of the IgM across the blood-CSF barrier.

Previous studies on OCMBs in MS suggested that intrathecal IgM belong to the *natural antibodies,* a subgroup of antibodies produced by CD5+ B lymphocytes. Such production is mainly driven by endogenous rather than non-self antigens (Lee et al., 2020), following antigen-driven affinity maturation without isotype switching (Beltran et al., 2014), Natural antibodies are likely to play a physiological role as first line of defense against infections, regulators of B cell development and autoimmunity, and promoters of the removal of cell debris (Maddur et al., 2020). Given their constitutional ability to bind self-antigens (Boes, 2000), natural IgM and CD5+ B lymphocytes could contribute to the pathogenesis of autoimmune diseases, including MS and systemic lupus erythematosus (Youinou et al., 1993; Seidi et al., 2002; Bohm, 2004).

In MS, at least part of the intrathecal natural IgM could recognize myelin-specific lipids (Villar et al., 2005; Bosca et al., 2010; Monreal et al., 2021), which likely act as antigenic triggers by inducing local somatic hypermutation within meningeal follicles, ultimately promoting a more aggressive disease (Boes, 2000; Berland and Wortis, 2002). Interestingly, however, natural IgM-producing CD5+ B lymphocytes are present in the peripheral circulation of pwMS too, and have been associated with a more aggressive disease course independent of the presence of OCMB pattern #2 (Villar et al., 2011). These data give biological plausibility to our findings of a worse prognosis in pwMS with OCMB pattern #4, with the understanding that the same holds for OCMB pattern #2. However, whether peripherally produced oligoclonal IgM pertain to the natural IgM subgroup has not been formally demonstrated so far and needs to be addressed in future studies.

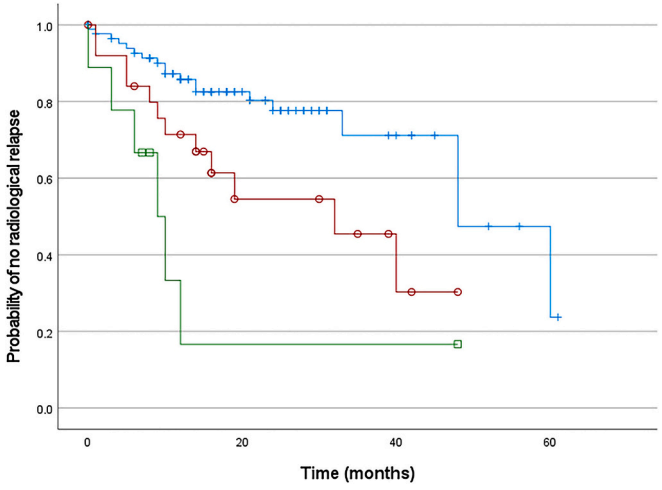

Fig. 2. Kaplan-Meier survival curves of pwMS without oligoclonal IgM bands (OCMBs; upper curve; blue crosses), with OCMBs that were identical in serum and CSF (pattern #4; middle curve; red circles), or with unique-to-CSF OCMBs (pattern #2; lower curve; green squares), on the probability of no radiological relapse over time. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

An important difference between our and other studies regards the frequency of OCMB pattern #2. Indeed, we found a lower frequency of this pattern (6.6 %) in comparison with what reported in the literature (i.e., from 23.2 % to 68.7 %) (Oechtering et al., 2021; Thangarajh et al., 2008; Pfuhl et al., 2019; Villar et al., 2002b; Perini et al., 2006), but with similar low percentages in the independent cohorts investigated in our two centers (i.e., 5.6 % and 8.6 %). The comparison of our findings with those reported by others is not easy, as OCMB pattern #4 seems to be not considered (Villar et al., 2003; Espino et al., 2015; Pfuhl et al., 2019), or, when reported (Espino et al., 2015; Villar et al., 2002b), not at all indicative of the "target abnormality", namely, and coherently, the intrathecal production of oligoclonal IgM.

The distinction between the two patterns refers to the well-established criteria for oligoclonal IgG band classification (Andersson et al., 1994). However, the recognition of pattern #4 on immunoblots is challenging, and yields large between-laboratory discrepancies in external quality control schemes for oligoclonal IgG bands, as it has long been recognized (Gastaldi et al., 2017; Franciotta and Lolli, 2007; Franciotta et al., 2005). The issue is basically unsolved, or very difficult to solve out, as shown in an interesting reproducibility study that involved three laboratories with long-lasting and established expertise in the field (Mariotto et al., 2021). Similar findings of rather large between-laboratory discrepancies have been reported in the only study on the assessment of reproducibility of OCMB detection (Espino et al., 2015). Besides, in another study the published pictures of IEF runs suggest that the distinction between pattern #2 and pattern #4 might mainly be due to a quantitative factor (Villar et al., 2001), and that, in some cases at least, pattern #2 could have turned into pattern #4 if the volume of the diluted serum, and therefore the amount of IgM, had been increased in the IEF run. It is a matter of fact that the best way to distinguish these two patterns, and definitely recognize them, is to run equal amounts of the tested immunoglobulin. This is recommended for oligoclonal IgG band determination (Andersson et al., 1994), but not included in the procedures for OCMB testing (Espino et al., 2015; Villar et al., 2001). Following these procedures is at risk of missing OCMB patterns #4, much to the advantage of patterns #2. We therefore suggest that the procedure of running equal amounts of serum and CSF IgM should be applied also to OCMB detection in the future.

5. Conclusions

In conclusion, the determination of OCMBs might work as a prognostic biomarker able to personalize patient management in MS, allowing the early identification of subjects who deserve more aggressive treatments (He et al., 2020). Of note, in both our two series, oligoclonal IgM were produced intrathecally less frequently than systemically, with rather similar prognostic value, but with the highest risk of radiological relapse in pwMS with the intrathecal IgM production. The meaning of such extra-CNS oligoclonal production holds potential and deserves future studies.

For a practical point of view, however, ten years after the experts' opinion that OCMBs were ready for clinical implementation (Teunissen et al., 2015), there are no prospective studies reporting real-life data on routine OCMB detection in unselected large series of pwCIS/MS with sufficiently long clinical follow-up. Moreover, as herein pointed out, laboratory procedures for OCMB detection are not standardized, complex, and time-consuming. Standardized, automated, lower-cost tests for the detection of promising biomarkers of MS prognosis, such as serum neurofilament light chain and glial fibrillary acidic protein (Bauer et al.,

2024; Monreal et al., 2024), will be likely used as an alternative to OCMBs in the near future.

CRediT authorship contribution statement

Francesco Masi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sabrina Al Qudsi:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Davide Visigalli:** Writing – review & editing, Methodology, Formal analysis. **Elisabetta Zardini:** Writing – review & editing, Methodology, Formal analysis. **Elisabetta Capello:** Writing – review & editing, Resources. **Luca Pio Dicembre:** Writing – review & editing. **Elena Colombo:** Writing – review & editing, Resources. **Antonio Uccelli:** Writing – review & editing, Resources. **Matteo Gastaldi:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Matilde Inglese:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Diego Franciotta:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2025.578622.

Data availability

The data that support the findings of this study are available on request from the corresponding author. DOI for the data: https://doi.org/10.5281/zenodo.14260516.

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