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Sleep Macrostructure, Cyclic Alternating Pattern and CSF Cytokines in De Novo Relapsing–Remitting Multiple Sclerosis: A Controlled Polysomnographic Study

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ABSTRACT

Multiple sclerosis is frequently associated with sleep disorders. This study aimed to evaluate subjective and objective sleep parameters in de novo relapsing–remitting multiple sclerosis patients compared to healthy controls and to explore correlations with cerebrospinal fluid cytokines. Twenty-one patients underwent CSF cytokine analysis, overnight polysomnography and sleep quality assessments; 21 HCs also participated in sleep evaluations. We analysed sleep macrostructure and the cyclic alternating pattern. Compared to controls, patients exhibited increased sleep period time, time in bed, REM sleep latency, N3 sleep percentage and wakefulness after sleep onset, along with reduced sleep efficiency and REM sleep percentage. These alterations were more pronounced in patients who also had obstructive sleep apnoea. Cyclic alternating pattern analysis revealed increased CAP time and rate—particularly higher A3 indices and duration of A phases—suggesting enhanced sleep fragmentation. A significant association was found between IL-1β and longer Phase B duration, and IL-15 showed a positive correlation with A3 mean duration in the cyclic alternating pattern, indicating inflammatory modulation of sleep architecture. Fatigue was negatively correlated with A1 mean duration and cycle mean duration. These findings demonstrate that de novo relapsing–remitting multiple sclerosis is associated with significant sleep fragmentation, particularly in patients with obstructive sleep apnoea and suggest that pro-inflammatory cytokines might influence sleep homeostasis through adaptive mechanisms. This underscores the importance of addressing sleep quality in multiple sclerosis management.

1 | Introduction

Multiple sclerosis (MS) is a chronic inflammatory immune-mediated disease considered one of the leading neurological causes of disability in young people (Thompson, Baranzini, et al. 2018). The clinical course of MS reflects the complex pathogenesis characterised by inflammation and neurodegeneration already detectable in the early stages of the disease. The interaction between these two processes is critically regulated by the expression of specific inflammatory mediators

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and neurotrophins (Mandolesi et al. 2015). Pro-inflammatory cytokines released by autoreactive lymphocytes and activated immune cells drive neuroinflammation and the development of white matter demyelinating lesions. Elevated cerebrospinal fluid (CSF) levels of specific pro-inflammatory cytokines have been associated with worse long-term disability and increased neurodegeneration in MS patients (Rossi et al. 2014, 2012; Stampanoni Bassi et al. 2020). Fatigue, depression, sleep disorders and cognitive decline are considered among the most severe symptoms, affecting more than 50% of patients (Kaynak et al. 2006; Sakkas et al. 2019). Among sleep disorders, insomnia may affect more than 20% of MS patients (Zeng et al. 2023), reported significantly more often by people with MS (PwMS) than by healthy individuals (Rzepka, Chmiela, Kaczmarczyk, and Krzystanek 2024). These common symptoms are often interrelated, further impacting the patient's overall well-being (Yeni et al. 2025). Poor sleep quality and daytime sleepiness could have a negative impact on patients' health and quality of life that further contributes to the burden of the disease (Amtmann et al. 2018; Hughes et al. 2018; Riccitelli et al. 2021). Furthermore, emerging evidence suggests a deeper, bidirectional relationship between sleep disturbances and MS pathophysiology, potentially influencing disease risk and clinical outcomes (Aoun et al. 2024; Cordone et al. 2025). Sleep disorders in MS have multifactorial aetiology and are significantly dependent on the severity of the disease (Morris et al. 2018). Although there are few polysomnographic data regarding PwMS (Zhang et al. 2023), recent polysomnographic data confirmed sleep fragmentation related to disability and cognition (Rzepka, Chmiela, Galus, et al. 2024; Rzepka, Chmiela, Kaczmarczyk, and Krzystanek 2024) and poorer subjective sleep quality (Moradi et al. 2025) and no studies regarding cyclic alternating pattern (CAP) and CSF cytokines in drug-naïve people with relapsing remitting MS (RRMS). The aim of our study was to investigate the sleep profile using subjective scales (Pittsburgh Sleep Quality Index [PSQI], Epworth Sleepiness Scale [ESS]), and polysomnographic data (sleep macrostructure and CAP) and a large panel of CSF cytokines in a homogeneous group of drug-naive RRMS patients.

2 | Materials and Method

2.1 | Patients and Controls

We prospectively evaluated a group of 21 consecutive patients admitted to the Neurology Department of Neuromed Hospital (Pozzilli, Italy) between 2018 and 2020. Patients were admitted for diagnostic purposes including neurological evaluation, brain and spinal cord MRI and CSF analysis. Inclusion criteria were (1) the subsequent confirmation of a Relapsing–Remitting Multiple Sclerosis (RRMS) diagnosis based on clinical, laboratory and MRI parameters according to the 2017 revised McDonald criteria (Thompson, Banwell, et al. 2018); (2) no other major systemic, psychiatric or neurological disorders; (3) absence of clinical relapse within the 90 days prior to evaluation; (4) absence of gadolinium-enhancing lesions on the diagnostic MRI scan. All patients were treatment-naïve for MS-specific therapies at the time of the study assessments; corticosteroids or disease-modifying therapies were initiated later as clinically indicated.

MS disease onset was defined as the first episode of focal neurological dysfunction indicative of MS, and disease duration was estimated from onset to diagnosis. Disability was assessed by a trained and certified neurologist using the Expanded Disability Status Scale (EDSS). Crucially, no patients were treated with corticosteroids or other MS-specific immunoactive therapies before CSF withdrawal and polysomnographic evaluation; such treatments were initiated later if appropriate. A group of 21 healthy controls (HCs), free from neurological or sleep disorders and similar in age, gender and BMI, were recruited for subjective sleep scale assessment and polysomnography. The study was approved by the local Ethics Committee (CE numbers 06/17 and 11/17), and all subjects provided written informed consent. The following exclusion criteria were used for patients and controls: (1) drug or substance abuse, (2) use of drugs known to significantly interfere with sleep architecture or CNS function (e.g., benzodiazepines, Z-drugs, specific antidepressants or antipsychotics, stimulants, opioids) within 4 weeks; (3) autoimmune and inflammatory disease other than RRMS; (4) clinically relevant medical or surgical conditions; (5) HCs: sleep disorders based on clinical history and polysomnography.

2.2 | CSF Collection and Analysis

In all patients, lumbar puncture was performed the day after PSG. Concentrations of cytokines in CSF were calculated according to a standard curve for the specific target and expressed in picograms/millilitre (pg/mL). The CSF chemokines examined included a pro-inflammatory panel (interleukin IL-1β, IL-2, IL-6, IL-8, TNFα, IL-17, IL-12 and IL-15) and an anti-inflammatory panel (IL-4, IL-10, IL-1 receptor antagonist [IL-1ra] and platelet-derived growth factor [PDGF]).

2.3 | Polysomnography

The subjects underwent an adaptation night in the laboratory for acclimatisation, which was not recorded for analysis (Veauthier et al. 2018). All reported polysomnographic data were obtained from the subsequent second night recording. Patients were also instructed to maintain the usual sleep schedule during the week preceding the evaluation, fill in a sleep log focusing on the light 'off' time, subjective sleep onset, light 'on' time, sleepiness and voluntary napping diurnal sudden sleep episodes. Analysis of the sleep logs confirmed patients maintained their habitual sleep schedules in the week preceding the evaluation, with no significant acute sleep deprivation reported immediately prior to the PSG night. Recordings were performed through a 32-channel system polygraph (SOMNOSCREEN SOMNOMEDICS, Germany). Electrodes were positioned according to the 10–20 International System. Montage consisted of two oculographic channels, three electromyographic channels (mental and anterior tibialis muscles), eight referential EEG channels (F3, C3, F4, C4, O1, O2, A1, A2), monitoring of oro-nasal airflow, respiratory effort (thoracoabdominal belts), EKG, oximetry and snoring. Sleep analysis was performed according to AASM criteria (Berry et al. 2012; Silber et al. 2007). Periodic Limb Movement Index (PLMI) and apnoea–hypopnoea index (AHI) (Berry et al. 2012) were scored under standard criteria (apnoea drop ≥ 90% for ≥ 10 s; hypopnoea drop by ≥ 30%, for ≥ 10 s in association with

either ≥ 3% arterial oxygen desaturation or an arousal), AHI was considered an exclusion criterion if > 15/h, in HCs. The scoring of the macrostructure was visually conducted using Domino 3.0 (SOMNOmedics Germany 2016) by AR, a neurologist board certified in neurology, neurophysiology and sleep medicine; he was blind regarding patient identity and group. The following sleep parameters were obtained: total sleep period (TSP; length of time from sleep onset to wake up), total sleep time (TST; total time in bed [TIB] minus sleep latency and time spent in wakefulness after sleep onset [WASO]; min); sleep efficiency (SE; TST divided by bed time × 100; %); TIB; sleep onset latency (SOL; measured from light off to the first sleep epoch, min); first REM latency (measured from the first epoch of sleep to the first epoch of REM sleep); WASO (min); number of awakenings per hour (AWN/h); number of phase shifts per hour (SS/h); N1 stage (expressed as a percentage of TST %); N2 (expressed as a percentage of TST %); N3 (expressed as a percentage of TST %); REM sleep (expressed as a percentage of TST). PSG files were then exported in European Data Format (EDF) and the visual CAP detection and analysis were performed.

The scoring of the CAP was computed using Hypnolab software (*SWS soft 1.2.236*). CAP visual scoring was analysed according to standard criteria (Ferri et al. 2005; Terzano et al. 2001). Throughout the night, CAP periods were identified, with Phase A subtypes (A1, A2, A3) and B, as well as non-CAP periods. The following CAP parameters were obtained: total CAP time (time spent in CAP), CAP rate (CAP rate, time spent in CAP/total NREM time × 100), CAP rate for each NREM phase (N1, N2, N3); percentage and average duration of subtypes A1, A2 and A3, indices A1, A2 and A3 (respectively the number of A1, A2 and A3 per hour), the mean duration of Phases A and B, mean duration of cycles and sequences, number of CAP sequences and average number of cycles per sequence (Romigi et al. 2022).

2.4 | Subjective Scales

Patients completed questionnaires about fatigue (Modified Fatigue Impact Scale [MFIS]) (Flachenecker et al. 2002; Kos et al. 2005), Fatigue Severity Scale (FSS) (Ottonello et al. 2016), depression (The Beck Depression Inventory-II, BDI-II) (Beck et al. 1996), sleep quality (PSQI) (Buysse et al. 1989; Curcio et al. 2013), insomnia (Insomnia Severity Index [ISI]) (Bastien et al. 2001; Castronovo et al. 2016), daytime sleepiness (ESS) (Johns 1991; Vignatelli et al. 2003). HC performed PSQI, ESS and ISI.

2.5 | Statistical Analysis

Statistical analysis of sleep parameters comparing RRMS patients with HCs was performed by a non-parametric test for independent Mann–Whitney samples. The chi-squared test was applied for categorical variables. Results were presented as the mean and standard deviation. A *p*-value below 0.05 was considered statistically significant. The 'obstructive sleep apnoea (OSA)' and 'No-OSA' groups were compared with each other and with the control group by the Kruskal–Wallis non-parametric ANOVA assay with effect size assessment (ε^2). When the relationship was significant ($p < 0.05$), Dwass-Steel-Critchlow-Fligner (DSCF) ($p < 0.05$ considered significant) was performed as a post hoc test. Partial correlation analysis (Spearman test) adjusted for age, gender, disease duration was performed between fatigue scales (FSS, MFIS), CK and sleep variables (Spearman rank correlation). For multiple comparisons, Benjamini–Hochberg (B–H) correction was applied to the *p* value. The Jamovi software was used for statistical analysis (vers. 2.3) (Şahin and Aybek 2019).

3 | Results

3.1 | Clinical Data

Twenty-one RRMS patients were selected. We selected 21 HC subjects similar in age, gender and body mass index (see Table 1).

3.2 | Subjective Scales of Sleep and Daytime Sleepiness

Patients in the RRMS group showed a significantly higher mean PSQI score than HC ($p < 0.001$) and a higher number of subjects with PSQI > 5 (RRMS 10/21 vs. HC 1/21 $p = 0.005$). Similarly, the mean ISI score was significantly higher in patients ($p = 0.004$) than in controls as well as the number of subjects with RRMS with a score > 8 (RRMS 6/21 vs. HC 0/21 $p = 0.02$). Notably, almost half (47.8%) of the RRMS patients reported poor sleep quality (PSQI > 5), and over a quarter (28.5%) reported clinically significant insomnia symptoms (ISI > 8), rates significantly higher than those observed in HCs. Restless legs syndrome (RLS) symptoms were reported in 3/21 patients (14.28%). There were no significant differences in the ESS mean score and the number of subjects with a pathological score (> 10) (Table 1).

3.3 | Polysomnographic Data (RRMS vs. HC)

Assessment of macrostructural polysomnographic variables showed that, compared to HCs, RRMS patients exhibited significant alterations in sleep architecture, indicative of increased sleep fragmentation. Key findings included markedly lower sleep efficiency (79.6% vs. 90.7%, $p < 0.001$), a fourfold increase in WASO (101.8 vs. 25.6 min, $p < 0.001$), and a significant reduction in REM sleep percentage (17.4% vs. 26.9%, $p < 0.001$). Interestingly, patients also showed a significant increase in N3 sleep percentage (27.1% vs. 19.9%, $p = 0.003$). Full details of all parameters are provided in Table 2. Sleep disordered breathing events were scored according to AASM criteria (Berry et al. 2012), differentiating obstructive, central and mixed types. No central sleep apnoea was identified in the cohort. In our cohort, 8 of 21 patients (38.1%) had an AHI > 5/h and were classified into the RRMS-OSA group. Of these eight patients, six presented with mild OSA (AHI 5–15/h) and two with moderate OSA (AHI > 15/h).

3.4 | Subjective Scales of RRMS Patient Group

RRMS subjects were divided into two groups: 'OSA' group (AHI > 5/h) and 'No-OSA' group (AHI < 5/h). We did not find

TABLE 1 | Demographic features and sleep scales in patients and healthy controls.

	HC ($N=21$)		RRMS ($N=21$)		HC vs. RRMS
	Mean	**SD**	**Mean**	**SD**	***p***
Age (year)	36.6	7.75	35.6	12.9	0.77
Gender (male/female)	9/12		9/12		$\chi^2=0$ $p=1$
BMI	25.59	3.15	26.2	4.6	0.73
PSQI global score	2.19	1.03	5.6	3.3	<0.001*
PSQI > 5, *n* (%)	1/21 (4.7%)		10/21 (47.8%)		$\chi^2=7.8$ $p=0.005$*
ESS	4.2	2.27	5.3	2.9	0.45
ESS > 10, *n* (%)	0/21 (0%)		1/21 (4.7%)		$\chi^2=1.02$ $p=0.31$
ISI	3.57	2.09	6.68	2	0.004*
ISI > 8, *n* (%)	0/21 (0%)		6/21 (28.5%)		$\chi^2=4.8$ $p=0.02$*

Note: Comparison between HC and RRMS by *T* test. $p<0.05$ is considered significant; χ^2 was applied for dichotomized data.
Abbreviations: BMI, body mass index; ESS, Epworth Sleepiness Scale; HC, healthy controls; ISI, Insomnia Severity Index; PSQI, Pittsburgh Sleep Quality Index; RRMS, relapsing remitting multiple sclerosis.
*Statistically significant value $p < 0.05$.

statistically significant differences in PSQI (sleep quality) scores; ESS (daytime sleepiness); ISI (insomnia); FSS; EDSS (disability scale); MFIS; BDI (depressive symptoms) between the two groups (Table 3). However, it should be noted that the RRMS-OSA group showed a trend towards worse outcomes in several measures, including PSQI ($p=0.08$), FSS ($p=0.1$) and MFIS ($p=0.15$). The lack of statistical significance may be attributable to the limited sample size of the subgroups.

3.5 | Macrostructural Polysomnographic Data (OSAS, No-OSA and HC Comparison)

3.5.1 | OSA vs. No-OSA

No macrostructural differences in sleep were detected in the group of patients with (OSA) and without sleep apnoea (No-OSA). The only significantly larger variables were AHI ($p<0.001$) and ODI ($p<0.001$).

3.5.2 | No-OSA vs. HC

In the group of patients without sleep apnoea (No-OSA) compared to controls, we found significantly reduced sleep efficiency ($p=0.01$), increased N3% sleep ($p=0.005$), reduced REM sleep % ($p<0.001$), longer WASO (min) ($p=0.002$) (Table 2).

3.5.3 | OSA vs. HC

The OSA RRMS group compared to HC showed a longer TSP ($p=0.04$), longer TIB ($p=0.01$), lower SE ($p=0.02$), higher REM sleep latency ($p=0.008$), lower REM sleep % ($p=0.001$), longer WASO (min) ($p=0.001$) and a higher number of phase shifts ($p=0.02$), greater AHI ($p<0.001$) and ODI ($p<0.001$) (Table 2).

3.6 | Sleep Microstructure: Cyclic Alternating Pattern (Comparison Between RRMS vs. HC)

Evaluation of microstructural variables revealed a profound increase in NREM instability in RRMS patients. Compared to HCs, patients had a markedly higher CAP rate (52.5% vs. 37.7%, $p<0.001$) and increased total CAP time (167.1 vs. 111.9 min, $p<0.001$). This was driven by a higher percentage of A3 phases (15.0% vs. 9.4%, $p=0.003$), longer mean duration of all A phase subtypes (A1, A2 and A3; all $p<0.001$), and a shorter duration of the B phases (23.6 vs. 26.9 s, $p<0.001$), indicating a more fragmented and unstable sleep process (Table 4).

3.7 | Sleep Microstructure: Cyclic Alternating Pattern

3.7.1 | OSA vs. HC

The OSA group when compared with HC showed higher CAP rate ($p=0.002$), CAP time ($p=0.003$), CAP rate in N1 ($p=0.005$), CAP rate in N2 ($p=0.001$), greater A3% ($p=0.003$), longer A1 duration ($p=0.039$), A2 ($p=0.006$) and A3 ($p=0.011$), higher index of A3 ($p<0.001$), longer Phase A duration ($p=0.004$), shorter duration of Phase B ($p=0.012$), higher number of sequences ($p=0.004$) (see Table 4).

3.7.2 | No-OSA vs. HC

Comparing No-OSA and HC, we found a higher CAP rate ($p<0.001$), increased CAP time ($p<0.001$), CAP rate in N2 ($p<0.001$), longer A1 ($p=0.001$), A2 ($p=0.002$) and A3 ($p=0.003$), a higher A1 index ($p<0.001$) and A3 ($p<0.001$), longer duration of Phase A ($p<0.001$), shorter duration of Phase B ($p<0.001$), longer duration of sequences ($p=0.048$), higher number of sequences ($p=0.03$) and cycles in sequence ($p=0.044$) (Table 4).

TABLE 2 | Comparison of conventional polysomnographic parameters in normal healthy controls (HC) and MS patients (RRMS; RRMS-OSA and RRMS No-OSA).

	HC		Patients						Mann–Whitney[a]		One-way non-parametric ANOVA Kruskal–Wallis[b]				
	HC ($N=21$)		RRMS ($N=21$)		RRMS-OSA ($N=8$)		RRMS No-OSA ($N=13$)		HC vs. TOT		Kruskal–Wallis		DSCF pairwise comparison (p)		
PSG variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p	Effect size (r)	p	ε^2	OSA vs. No-OSA	No-OSA vs. HC	OSA vs. HC
SOL (min)	13.5	11.2	20.9	36.2	31.2	57.3	14.5	12.4	0.9	0.02	0.95	0.002	0.96	0.96	0.99
TST (min)	403.1	56.99	393.76	79.44	416.3	114.17	380	48.6	0.58	0.1	0.38	0.004	0.52	0.46	0.85
SPT (min)	428.74	53.7	475.29	85.8	496	114.07	463	65	0.03*	0.39	0.04	0.15	0.35	0.4	*0.04*
TIB (min)	443.57	51.4	496.7	71.8	527.3	68.56	478	69.7	0.0019*	0.42	0.02	0.19	0.31	0.39	*0.01*
SE (%)	90.7	5.32	79.58	12.59	77.98	14.54	80.6	11.8	< 0.001*	0.61	0.003	0.28	0.9	0.01	0.02
REM LAT (min)	76.3	26.08	117.79	57.85	149.68	75.2	98.2	34.4	0.004*	0.52	0.004	0.26	0.17	0.12	0.008
NI%	7.25	1.82	10.15	5.85	11.15	6.9	9.54	5.32	0.29	0.2	0.56	0.02	0.9	0.6	0.74
N2%	45.8	5.72	45.2	5.81	46.16	8.3	44.7	3.88	0.48	0.12	0.45	0.03	0.61	0.47	0.96
N3%	19.95	5.03	27.13	9.82	26.5	13.49	27.5	7.35	0.003*	0.55	0.006	0.25	0.47	0.005	0.27
REM%	26.9	5.22	17.4	5.14	16.07	5.77	18.3	4.74	< 0.001*	0.82	< 0.001	0.50	0.7	< 0.001	0.001
WASO (min)	25.6	21.3	101.8	68.8	110.6	66.96	96.4	72.1	< 0.001*	0.77	< 0.001	0.44	0.83	0.002	0.001
AWK/h	3.4	1.81	4.8	3.15	5.96	4.05	4.09	2.35	0.16	0.254	0.25	0.06	0.68	0.69	0.24
SS/h	13.4	3.6	18.2	6.1	20.7	7.65	16.6	4.68	< 0.006*	0.497	< 0.001	0.76	0.43	0.11	0.02
PLMI	4.24	4.43	4.88	5.25	3.7	4.2	5.6	5.84	0.93	0.01	0.85	0.008	0.79	0.96	0.98
PLMI > 15/h, n (%)	1/21 (4.7%)		1/21 (4.7%)		0 (0%)				$\chi^2=0$ $p=1$						
AHI	2.96	1.67	6.9	13.3	15.41	19.2	1.68	1.19	0.75	0.05	< 0.001	0.52	< 0.001	0.05	< 0.001
AHI > 5/h, n (%)	1/21 (4.7%)		8/21 (38.1%)						$\chi^2=6.9$ $p=0.008$						
AHI > 15/h	0/21 (0%)		2/21 (9.5%)						$\chi^2=0.52$ $p=0.46$						
ODI	2.75	1.56	6.58	12.16	14.75	17.22	1.55	1.23	0.77	0.05	< 0.001	0.51	< 0.001	0.05	< 0.001

Abbreviations: AWK/h, number of awakenings per hour; REM LAT, REM latency; SE, sleep efficiency; SH/h, stage shifts per hour; SOL, sleep onset latency; TST, total sleep time; TIB, time in bed; WASO, wake after sleep onset in minutes.

[a]Comparison between HC and T0 by Mann–Whitney U test. $p < 0.05$ is considered significant.

[b]Comparison between HC, RRMS-OSA and RRMS No-OSA by Kruskal–Wallis non-parametric ANOVA. Dwass-Steel-Critchlow-Fligner (DSCF) pairwise comparisons post hoc test $p < 0.05$ is considered significant. The following sleep parameters were obtained: total sleep time (TST; total time in bed minus sleep latency and time spent in wakefulness after sleep onset; min); sleep efficiency (SE; total sleep time divided by bed time × 100; %); time in bed (TIB); sleep onset latency (SOL; measured from light off to the first sleep epoch, min); first REM latency (measured from the first epoch of sleep to the first epoch of REM sleep); wakefulness after sleep onset WASO (min); number of awakenings per hour AWN/h; number of phase shifts per hour SS/h; N1 stage (percentage of total sleep time %); N2 (percentage of total sleep time %); N3 (percentage of total sleep time %); REM sleep (percentage of total sleep time).

*Statistically significant value $p < 0.05$.

TABLE 3 | Comparison of demographic, clinical and subjective sleep scale data in RRMS patient subgroups (OSA vs. No-OSA).

	RRMS ($N=21$)		RRMS-OSA ($N=8$)		RRMS No-OSA ($N=13$)		OSA vs. No-OSA
	Mean	SD	Mean	SD	Mean	SD	*p*
Age (year)	35.6	12.9	39.38	14.49	33.4	11.84	0.31
Gender (male/female)	9/12		5/3		4/9		$\chi^2=0.94$ $p=0.33$
BMI	26.2	4.6	27.1	5.7	25.3	4.64	0.45
PSQI global score	5.6	3.3	7.25	3.45	4.69	2.95	0.08
PSQI > 5, *n* (%)	10/21 (47.8%)		6/8 (75%)		4/13 (30.7%)		$\chi^2=2.3$ $p=0.12$
ESS	5.3	2.9	6	2.73	5.08	3.12	0.45
ESS > 10, *n* (%)	1/21 (4.7%)		0/8 (0%)		1/13 (7.6%)		$\chi^2=0.64$ $p=0.42$
ISI	6.68	2	8.13	5.36	6.08	3.8	0.31
ISI > 8, *n* (%)	6/21		3/8		3/13		$\chi^2=0.04$ $p=0.83$
Disease duration (year)	1.13	1.71	1.36	2.4	0.98	1.2	0.63
EDSS	1.21	0.75	1.31	0.7	1.15	0.8	0.65
FSS	1.94	1.15	2.46	1.36	1.62	0.9	0.1
FSS > 4	1/21 (4.7%)		1/8 (12.5%)		0/13 (0%)		$\chi^2=0.06$ $p=0.80$
MFIS, 0–84	16.3	12.2	21.3	11.8	13.3	11.9	0.15
MFIS ≥ 38, *n* (%)	1/21 (4.7%)		1/8 (12.5%)		0/13 (0%)		$\chi^2=0.06$ $p=0.80$
BDI-II	8.1	6.83	6	4.87	9.46	7.68	0.27
BDI-II > 18	1/21 (4.7%)		0/8 (0%)		1/13 (7.6%)		$\chi^2=0.06$ $p=0.80$

Note: RRMS OSA MS patients with sleep apnoea; RRMS No-OSA MS patients without OSAS; Student's *t*-test or chi-squared test was used for dichotomized data; $p<0.05$ is considered significant.
Abbreviations: BDI-II, beck depression inventory II; BMI, body mass index; EDSS, Expanded Disability Status Scale; ESS, Epworth Sleepiness Scale; FSS, Fatigue Severity Scale; ISI, insomnia severity index; MFIS, Modified Fatigue Impact Scale; OSAS, obstructive sleep apnoea syndrome; PSQI, Pittsburgh sleep quality index.

3.7.3 | OSA vs. No-OSA

No microstructural differences in sleep were detected in RRMS OSA vs. No-OSA.

3.8 | Correlation Between Fatigue and PSG Parameters

We did not find any significant correlation between fatigue scales score (FSS, MFIS) and sleep macrostructural parameters. We found a significant negative association of FSS and the duration of Phase A1 of the CAP ($r=-0.67$, $p=0.0026$) (Figure 1A). MFIS showed a negative correlation with A1 mean duration ($r=-0.66$, $p=0.0053$) (Figure 1B) and cycle mean duration ($r=-0.69$, $p=0.0026$) (Figure 1C).

3.9 | Correlation Between Cytokines and PSG Parameters

We did not find any significant correlation between CSF CK and sleep macrostructural parameters. We found a significant positive association of IL-1β with the duration of Phase B of the CAP ($r=0.635$, $p=0.0075$) (Figure 2A), and negative correlation with sequence mean duration ($r=-0.62$, $p=0.005$) (Figure 2B) and number of cycles per sequence ($r=-0.65$, $p=0.005$) (Figure 2C). IL-15 showed a positive correlation with A mean duration ($r=0.64$, $p=0.004$) (Figure 3A) and A3 mean duration ($r=0.75$, $p=0.0026$) (Figure 3B).

4 | Discussion

To our knowledge, this is the first study to combine detailed macro- and micro-structural sleep analysis with CSF cytokine profiling in a cohort of newly diagnosed, treatment-naïve RRMS patients. Our results reveal that profound sleep disruption is present from the very early stages of RRMS, characterised by the co-existence of increased N3 sleep alongside high NREM sleep instability, as measured by CAP analysis. This seemingly paradoxical pattern—a homeostatic drive for deeper sleep occurring within a framework of profound fragmentation—suggests a state of compensatory homeostatic dysregulation. In this context, the increase in N3 sleep and A1-subtype CAP activity can be interpreted as an attempted, albeit inefficient, compensation by the brain to counteract the destabilising effects of the underlying disease process. However, the concomitant and marked increase in sleep fragmentation (i.e., the high overall CAP rate) indicates that this compensatory effort is ultimately overwhelmed, leading to the net effect of unrefreshing sleep that contributes to symptoms like fatigue. Interestingly, this polysomnographic signature

TABLE 4 | Comparison of cyclic alternating pattern parameters in normal healthy controls (HC) and MS patients (RRMS; RRMS-OSA and RRMS No-OSA).

	HC		RRMS						Mann–Whitney[a]		One-way non-parametric ANOVA Kruskal–Wallis[b]				
	HC ($N=21$)		RRMS ($N=21$)		RRMS OSA ($N=8$)		RRMS No-OSA ($N=13$)		HC vs. RRMS		Kruskal–Wallis		DSCF pairwise comparison (p)		
PSG variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p	Effect size (r)	p	Effect size ε^2	OSA vs. No-OSA	No-OSA vs. HC	OSA vs. HC
CAP rate (%)	37.67	7.40	52.51	7.15	51.38	6.89	53.22	7.49	<0.001*	0.8685	<0.001*	0.56926	0.83	<0.001*	0.002*
CAP time (min)	111.93	30.97	167.13	38.37	174.14	46.19	162.82	34.01	<0.001*	0.7642	<0.001*	0.44208	0.83	<0.001*	0.003*
CAP_rate N1 (%)	25.04	10.44	38.18	14.34	44.91	15.63	34.03	12.31	0.003*	0.5329	0.004*	0.27574	0.17	0.14	*0.005*
CAP_rate N2 (%)	26.09	8.57	49.59	11.31	49.74	13.75	49.49	10.15	<0.001*	0.9320	<0.001*	0.65208	0.97	<0.001*	*0.001*
CAP_rate N3 (%)	69.07	12.83	66.90	11.7	65.2	15.35	67.92	9.37	0.70	0.0703	0.924	0.00385	0.97	0.92	0.96
A1%	68.66	8.74	66.9	10.84	63.14	12.72	69.22	9.28	0.821	0.0431	0.470	0.03678	0.43	0.93	0.6
A2%	21.92	6.57	18.13	4.87	18.7	5.67	17.7	4.51	0.037*	0.3787	0.108	0.10873	1	0.1	0.41
A3%	9.41	3.63	14.98	6.72	18.15	7.5	13.03	5.62	0.003*	0.5329	0.002*	0.29912	0.1	0.18	0.003*
A1 duration	5.84	1.02	7.2	1.01	6.92	1	7.37	1.01	<0.001*	0.68	<0.001*	0.36713	0.5	0.001*	0.039*
A2 duration	6.56	1.61	9.2	1.94	9.57	2.16	8.96	1.83	<0.001*	0.7279	<0.001*	0.40150	0.81	0.002*	0.006*
A3 duration	7.79	1.86	10.47	2.25	10.9	3.07	10.19	1.66	<0.001*	0.6916	<0.001*	0.35965	0.98	0.003*	0.011*
A1 index (n/h)	36.65	8.7	48.17	9.13	44.2	10.05	50.61	7.95	<0.001*	0.6735	<0.001*	0.38180	0.21	<0.001*	0.081
A2 index (n/h)	9.74	4.24	12.6	4.6	13.04	4.78	12.32	4.66	0.068	0.3311	0.145	0.09431	0.74	0.4	0.16
A3 index (n/h)	3.16	1.44	8.64	4.45	10.53	4.22	7.48	4.34	<0.001*	0.8617	<0.001*	0.59133	0.17	<0.001*	<0.001*
A duration (s)	6.16	1.05	7.99	1.10	7.96	1.17	8.01	1.10	<0.001*	0.7823	<0.001*	0.45988	0.98	<0.001*	0.004*
B duration (s)	26.87	2.04	23.63	1.93	24.21	2.03	23.27	1.84	<0.001*	0.7800	<0.001*	0.46751	0.7	<0.001*	0.012*
Cycle mean duration (s)	33.06	2.17	31.59	2.26	32.1	2.74	31.28	1.27	0.057	0.34	0.142	0.09516	0.95	0.11	0.607
Sequences duration	214.05	43.29	244.76	44.51	233.64	39.65	251.6	47.45	0.02*	0.41	0.048*	0.14785	0.56	0.048*	0.442
CAP sequences (n)	32.29	8.19	42	7.26	45.88	8.53	39.62	5.41	<0.001*	0.61	<0.001*	0.34444	0.104	0.03*	0.004*
Cycles in sequences (n)	7.55	1.64	8.82	1.77	7.55	1.64	9.13	1.92	0.01*	0.42	0.045*	0.15169	0.61	0.044*	0.41

Note: CAP periods were identified, with Phase A subtypes (A1, A2, A3) and B, as well as non-CAP periods. The following CAP parameters were obtained: total CAP time (time spent in CAP), CAP rate (CAP rate, time spent in CAP/total NREM time × 100), CAP rate for each NREM phase (N1, N2, N3); percentage and average duration of subtypes A1, A2 and A3, indices A1, A2 and A3 (respectively the number of A1, A2 and A3 per hour), the mean duration of Phases A and B, mean duration of cycles and sequences, number of CAP sequences and average number of cycles per sequence.

Abbreviation: CAP, cyclic alternating pattern.

[a]Comparison between HC and RRMS by Mann–Whitney U test. $p < 0.05$ is considered significant.

[b]Comparison between HC, RRMS-OSA and RRMS No-OSA by Kruskal–Wallis non-parametric ANOVA. Dwass-Steel-Critchlow-Fligner (DSCF) pairwise comparisons post hoc test $p < 0.05$ is considered significant.

*Statistically significant value $p < 0.05$.

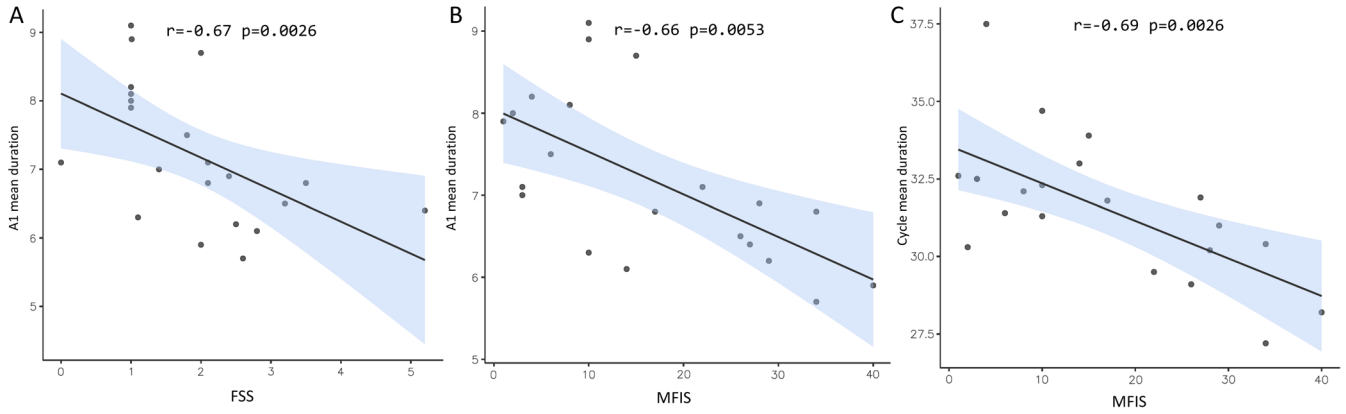

FIGURE 1 | Scatter plot with regression line and 95% confidence interval band, showing a negative correlation between A1 mean duration and FSS (A) and MFIS scores (B), and between cycle mean duration and MFIS (C).

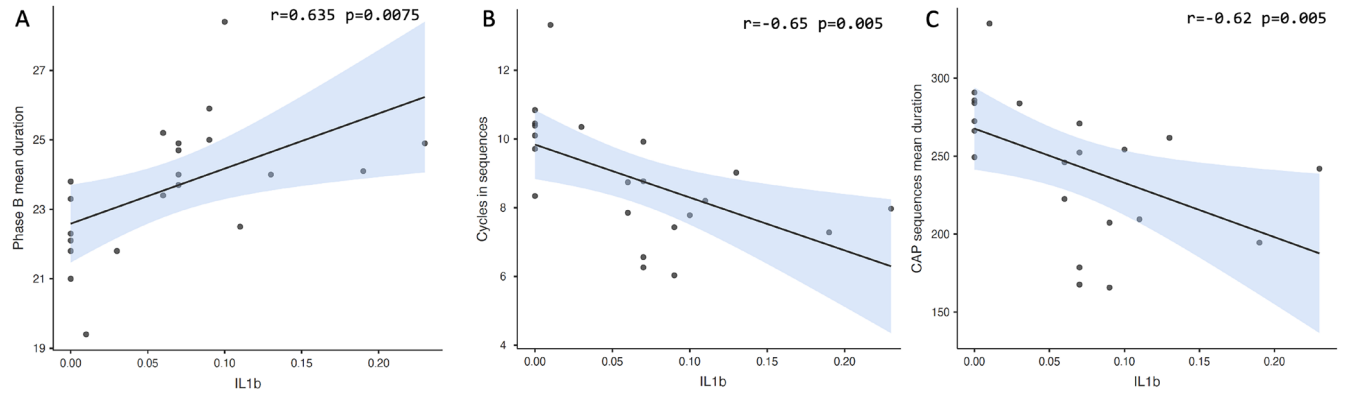

FIGURE 2 | Scatter plot with regression line and 95% confidence interval band, showing a positive correlation between Phase B mean duration and IL-1b (A) and a negative correlation between cycles in sequences (B), and CAP sequences mean duration and IL-1b (C).

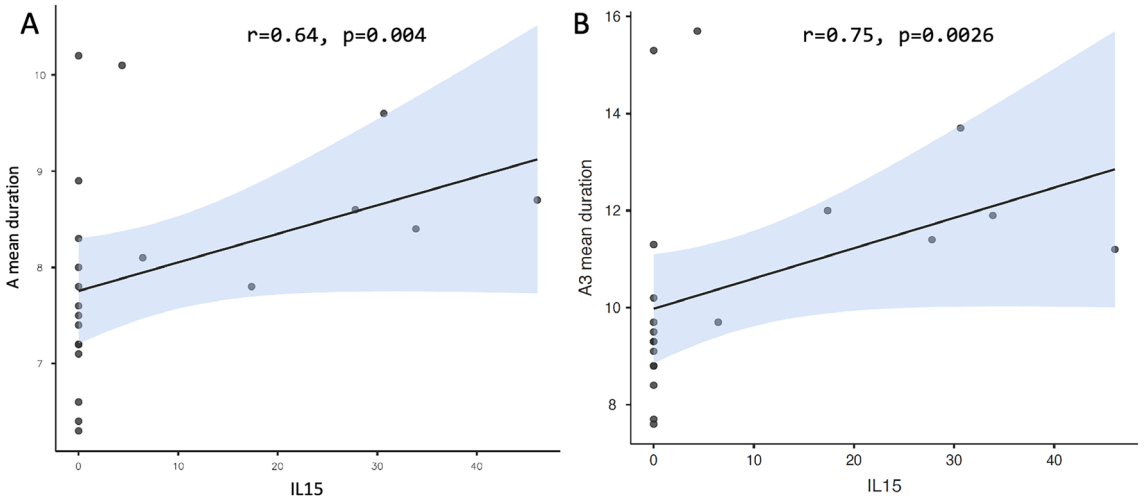

FIGURE 3 | Scatter plot with regression line and 95% confidence interval band, showing a positive correlation between A mean duration (A) and A3 mean duration and IL-15 (B).

is not exclusive to MS. A similar profile of high NREM sleep instability, as measured by CAP rate, concurrent with increased N3 sleep has been described in myotonic dystrophy type 1, a genetic neurodegenerative disorder (Bonanni et al. 2018). The presence of this shared pattern across two distinct central nervous system pathologies suggests it may represent a transversal marker of central sleep–wake dysregulation. Our findings also confirm the high prevalence of subjective sleep disturbances in de novo RRMS, in line with previous reports highlighting poor sleep quality and insomnia as early and common features of the disease (Brass et al. 2014; Moradi et al. 2025; Rzepka, Chmiela, Kaczmarczyk, and Krzystanek 2024). This underscores the

importance of screening for sleep disorders early in the disease course, especially given the strong interplay between sleep problems, fatigue and depression, which collectively impact quality of life (Yeni et al. 2025). The aetiology of these disturbances is multifactorial, involving anxious-depressive symptoms, fatigue, inflammatory processes and comorbid conditions (Braley 2017; Cordone et al. 2025) such as RLS and nocturia, which are themselves independent predictors of poor sleep in PwMS (Alis et al. 2025; Rzepka, Chmiela, Kaczmarczyk, and Krzystanek 2024). On the other hand, the presence of sleep disorders such as RLS seems to be associated with higher levels of fatigue (Riccitelli et al. 2021). In our cohort, we identified RLS symptoms in 3 out of 21 patients (14.3%), a prevalence that aligns with the wide range reported in MS populations. This comorbidity is highly relevant, as RLS is a well-established cause of significant sleep disruption. Very recently Alis et al. (2025) have emphasised that RLS is not merely correlated with poor sleep but is a strong independent predictor of it in the MS population, suggesting it acts as a primary driver of both poor sleep quality and the resulting fatigue. Furthermore, emerging evidence is uncovering a direct link between RLS and neuro-inflammatory processes. A recent transcriptomic analysis specifically implicated inflammation-related signalling pathways, such as the IL-17 pathway, in the pathophysiology of RLS (Mogavero et al. 2024). This supports the hypothesis that an abnormal immune response contributes to the syndrome, which is consistent with its higher prevalence in immune-mediated diseases like MS. Therefore, while the low number of RLS cases in our cohort prevents statistical analysis, it is plausible that for these patients, RLS acts as an additional driver of sleep fragmentation and fatigue, potentially compounding the effects of the primary MS pathology we have described. This highlights the clinical importance of screening for RLS in MS patients presenting with severe fatigue and sleep complaints. A key finding in our cohort was the high prevalence (38.1%) of OSA, confirming that sleep-related respiratory disorders are common in MS, potentially driven more by the disease process than traditional risk factors (Braley 2017). When analysing subgroups, the RRMS-OSA group exhibited intense sleep fragmentation without the compensatory increase in N3 sleep. In contrast, the RRMS No-OSA group showed less severe fragmentation but a significant increase in N3. Therefore RRMS-OSA shows greater sleep fragmentation, while the putative compensatory mechanism N3 seems evident only in the RRMS non-OSA group. An alternative, though not mutually exclusive, explanation is that the chronic low-grade inflammatory state inherent to MS may increase the tolerance to the physiological stressors of OSA, thereby blunting the differential impact on subjective and objective sleep measures that might be seen in a non-MS population. This may also explain the apparent paradox of poor subjective sleep quality (PSQI > 5) co-existing with an increased percentage of N3 sleep. The elevated N3 can be interpreted as a sign of a strong, possibly excessive, homeostatic drive for deep sleep, which is ultimately rendered unrefreshing by the profound underlying NREM instability. A single study has shown greater *arousability* (arousal index) in patients with MS and more evident in patients reporting fatigue (Kaynak et al. 2006).

The CAP represents a marker of both homeostatic processes preserving NREM sleep continuity and when altered represents an 'instability marker' of NREM (Parrino and Vaudano 2018; Parrino et al. 2014). The CAP analysis provides a deeper understanding of this NREM instability. The increased CAP rate in our patients confirms a greater fragmentation of sleep compared to controls. This was characterised by an increase in the duration of all A phase components and a reduction of Phase B duration, reflecting a dynamic between an adaptive, sleep-promoting response (A1) and a maladaptive, wake-promoting process (A3). The developing scenario reveals a blend of vulnerabilities and adaptive mechanisms, where the brain utilises CAP to maintain a dynamic equilibrium. However, the fragmentation mechanisms, evidenced by the increased A3% component and shorter Phase B duration, appear to prevail, predisposing patients to fatigue and cognitive dysfunction.

The prevalent mild-to-moderate OSA in our RRMS sample (mean AHI of 15.4 in the OSA group), with the majority of cases being mild (6/8 patients with AHI 5–15/h), could contribute to the greater fragmentation of NREM sleep (Gnoni et al. 2021).

The marked increase in CAP rate observed in our sample is consistent with findings in other neuro-inflammatory conditions like fibromyalgia and systemic sclerosis (Gök et al. 2025; Rizzi et al. 2004), and contrasts with the reduced CAP rate seen in some neurodegenerative diseases like Parkinson's Disease (Dagay et al. 2025). This divergence suggests that an elevated CAP rate might be a specific feature of sleep instability driven by neuroinflammation, whereas a reduced CAP rate could reflect a primary failure of arousal-generating systems common in neurodegeneration.

We also found a significant negative correlation between fatigue scales (FSS and MFIS) and the mean duration of CAP A1 and cycle duration. This provides objective PSG evidence linking reduced NREM sleep stability to higher perceived fatigue in de novo RRMS patients, suggesting that this instability is a significant contributor to MS-related fatigue from the earliest stages of the disease.

Finally, our study links these sleep microstructural alterations to central inflammation. While we found no correlations with sleep macrostructure, specific cytokines were associated with CAP parameters. The positive correlation between IL-1β and the duration of Phase B, a marker of NREM sleep stability, may appear counterintuitive. However, this can be interpreted in the context of the known dose-dependent, biphasic effects of IL-1β, where lower or chronic levels can be somnogenic (Besedovsky et al. 2019; Cordone et al. 2025). In the stable, early phase of RRMS, this may reflect an adaptive homeostatic response to consolidate sleep. Conversely, the negative correlations of IL-1β with CAP sequence parameters may reflect the concomitant destabilising aspect of this same cytokine, highlighting its complex dual role. It is also plausible that this relationship could be state-dependent. The observed stabilising effect of IL-1β on Phase B duration might be characteristic of the early disease stage, whereas in more advanced or progressive stages of MS, chronic inflammation could lead to a reduction in the sleeping brain to oscillate. This hypothesis should be explored in future longitudinal studies. The correlation of IL-15 with A3 mean duration further underscores the potential of inflammatory mediators to influence sleep architecture (Besedovsky et al. 2019; Romigi and Maestri 2014). Furthermore, our findings should be interpreted in the context of the evolving understanding of cytokines not merely as inflammatory mediators, but as direct

neuromodulators. Cytokines can influence a wide range of neural processes (Martins and Harrison 2025), including neurotransmitter function, synaptic plasticity, and the regulation of cortical sleep states. This perspective suggests that the correlations we observed between specific cytokines (IL-1β, IL-15) and sleep microstructure may reflect a direct modulatory impact on the neural circuits governing sleep stability, rather than being solely a downstream consequence of inflammation.

In conclusion, the sleep alterations in RRMS patients highlight the need for comprehensive sleep assessments. The interactions between sleep disturbances and inflammatory processes suggest that managing sleep quality may not only improve quality of life (Yeni et al. 2025) but also potentially modulate symptom severity and disease activity (Aoun et al. 2024). Additionally, longitudinal studies could elucidate the causal relationships between sleep changes and disease progression in RRMS.

5 | Strengths and Limitations

This study has strengths, including the recruitment of a well-characterised cohort of de novo, drug-naïve RRMS patients, the use of objective PSG with detailed CAP analysis alongside subjective scales, the inclusion of CSF cytokine analysis, and the comparison with a matched HC group. However, some limitations should be acknowledged. Firstly, while patients were categorised based on AHI (> 5/h for OSA group), the potential independent impact of even mild-to-moderate OSA on sleep architecture and related symptoms (such as fatigue or sleepiness) must be considered when interpreting comparisons between the RRMS-OSA, RRMS-No-OSA and HC groups. OSA itself can cause sleep fragmentation and daytime symptoms, which may overlap with or worsen those related directly to MS, potentially confounding the results attributed solely to the disease process. Secondly, although MRI scans were performed as part of the diagnostic workup, this study did not include quantitative analyses of MRI metrics, such as lesion load or specific lesion locations, nor were correlations performed between such MRI data and the measured sleep parameters. Incorporating detailed radiological data could provide further insights in future studies. The primary limitation of this study is its relatively small sample size ($N = 21$), which, when divided into subgroups, restricts statistical power. This is particularly relevant for the comparisons between the RRMS-OSA and RRMS-No-OSA groups, where the lack of statistically significant differences in subjective scales could represent a type 2 error. Consequently, these subgroup findings should be interpreted with caution. Secondly, the cross-sectional design limits the ability to draw definitive conclusions about causality or the evolution of sleep disturbances over the course of the disease; longitudinal studies are needed to address these aspects.

Author Contributions

A. Romigi: conceptualization, methodology, software, investigation, formal analysis, supervision, writing – original draft, writing – review and editing. **M. Stampanoni Bassi:** methodology, data curation. **M. Caccamo:** methodology, formal analysis, data curation, investigation, software. **L. Gilio:** methodology, formal analysis, data curation, investigation. **F. Ceriello:** validation, visualization, supervision. **A. Finardi:** methodology, data curation, investigation, supervision. **G. Vitrani:** validation, visualization, supervision. **I. Rosenszweig:** supervision, project administration, writing – review and editing. **R. Furlan:** investigation, methodology, supervision. **F. Buttari:** conceptualization, methodology, supervision, writing – review and editing. **D. Centonze:** conceptualization, funding acquisition, methodology, supervision, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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