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ORIGINAL ARTICLE

Association between CD20$^+$ T lymphocytes and neuropsychological findings in multiple sclerosis

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Abstract

Background and purpose: CD20$^+$ T lymphocytes are a subset of circulating T cells presenting the CD20$^+$ receptor, a molecular marker of B lineage. CD20$^+$ T lymphocytes are thought to play a pivotal role in multiple sclerosis (MS) pathology, especially at progressive stages. We aimed to investigate the correlation between CD20$^+$ T lymphocytes and neuropsychological features (i.e., cognition, depression, anxiety, fatigue, and sleep quality) in MS patients.

Methods: We enrolled 90 MS patients. Each patient underwent cognitive assessment (Brief International Cognitive Assessment for Multiple Sclerosis) and psychometric assessment (modified Fatigue Impact Scale, Beck Anxiety Inventory, Beck Depression Inventory, Pittsburgh Sleep Quality Index). Cognitive status was defined through the cerebral functional score.

Results: Forty-four of 90 patients were relapsing–remitting (49%) and 46 were progressive patients (51%). Seventy patients (18.9%) showed CD20$^+$ T lymphocytes in peripheral blood with a mean level of $0.38 \pm 1.2\%$. Patients with CD20$^+$ T lymphocytes were more likely to be at progressive phases (76.5% vs. 23.5%, $p = 0.02$) and showed a higher Expanded Disability Status Scale score (median [range] $= 6.0$ [1.5–7.5] vs. 3.5 [1–7.5], $p = 0.001$). Moreover, patients with CD20$^+$ T lymphocytes showed worse cognitive functioning ($p = 0.004$), higher global fatigue symptoms ($p = 0.02$), higher cognitive fatigue ($p = 0.01$), higher psychosocial fatigue ($p = 0.005$), and a trend toward worse sleep quality ($p = 0.06$).

Conclusions: The presence of CD20$^+$ T lymphocytes in the peripheral blood of MS patients was associated with worse neuropsychological functioning and progressive disease stages. Peripheral CD20$^+$ T lymphocytes could potentially serve as markers for both disease progression and development of fatigue in MS patients.

KEYWORDS
CD20$^+$ T lymphocytes, compartmentalized inflammation, fatigue, multiple sclerosis, neuropsychological

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory–neurodegenerative disease of the central nervous system (CNS) [1]. Pathological mechanisms underpinning MS are quite heterogeneous and result in white matter demyelination, neuroaxonal disruption, and loss [2]. Such pathological alterations are fueled by the activation of immune cells circulating in the blood as well as by immune cells resident within the CNS [3]. Various types of lymphocytes, including different subsets of T cells (such as $CD4^+$, $CD8^+$, $CD3^+CD20^+$, and regulatory T cells), B cells (including naive and memory B cells, and naive and memory B regulatory cells), and natural killer cells, play a key role in MS pathology [3, 4]. The precise interaction among these lymphocyte remains elusive, but it has become clear that CNS compartmentalized inflammation sustains chronic inflammatory–degenerative processes [4].

In a recent report, a subset of T cells expressing B cell markers ($CD20^+$ T lymphocytes) has been reported to be elevated in the peripheral blood of MS patients especially at progressive phases [5]. As regards this concept, it was observed that either $CD20^+$ cells, $CD4^+$ T cells, and $CD8^+$ T cells or IL-17^+ cells are present in chronic lesions in MS patients [6].

Consequently, this would suggest that $CD20^+$ T lymphocytes might reflect the activation of CNS compartmentalized inflammation.

From the pathological point of view, the different burden of the specific pathological processes reflects the heterogeneous clinical manifestation (i.e., motor disability, depressive symptoms, sexual dysfunction, etc.) [7].

Cognitive impairment and fatigue are the most common non-motor symptoms of MS. Fatigue affects up to 83% of patients with MS, significantly influencing the quality of life [8]. Cognitive impairment impacts 40%–65% of MS patients and plays a significant role in determining their level of functioning in daily activities. Cognitive impairment can manifest at the early stages involving deficits in long-term memory, executive function, attention, concentration, information-processing efficiency, and processing speed [9].

The exact mechanisms triggering and sustaining fatigue along the disease course remain elusive. Different magnetic resonance imaging (MRI)-derived measures such as lesion burden, brain atrophy, lesion locations, brain network derangement, and deficits in neurotransmitter-dependent cortical activity have been associated with fatigue, without a clear-cut causative association [8, 10]. Recent evidence indicates distinctive patterns of changes in monoaminergic networks over time, depending on the fatigue status of individuals with MS. These findings suggest a unique involvement of networks and brain regions in MS, which corresponds to the concurrent development of fatigue symptoms [10, 11].

Adding to the complexity, fatigue manifests as a multifaceted symptom encompassing different domains, including physical fatigue, cognitive fatigue, and psychosocial fatigue [8].

Given the correlations between $CD20^+$ T lymphocytes and chronic lesion [5] of the CNS, as well as between this type of lesion and the development of fatigue [12], there may be a potential connection between the extent of circulating $CD20^+$ T lymphocytes and fatigue.

In line with this hypothesis, we performed a cross-sectional study aiming to assess $CD20^+$ T lymphocytes in peripheral blood samples of MS patients and to investigate their correlation with fatigue. Furthermore, we aimed to study a possible link between $CD20^+$ T cells and cognitive impairment in MS patients, as well as a potential involvement in the development of neuropsychological disorders such as depression, anxiety, and sleep disorders.

EXPERIMENTAL PROCEDURES

Study design and population

This was a single-center cross-sectional study including consecutive MS subjects enrolled at the MS Clinical Care and Research Center of the Federico II University Hospital of Naples, Italy, satisfying the following inclusion criteria: (i) MS diagnosis according to the 2017 McDonald criteria [13]; (ii) no history of significant medical illnesses, fever, or substance abuse in the 30 day before assessment; (iii) no other major systemic, psychiatric, or neurological diseases; (iv) no relapse or corticosteroid treatment in the 30 days before assessment; and (v) stable on disease-modifying treatment (DMT) for at least 6 months preceding sample collection (i.e., adherence to treatment without treatment change or dose adjustment).

Standard protocol approvals, registrations, and patient consents

Approval was received from the local ethical committee Comitato Etico Campania 3 (C33539003). All subjects gave written informed consent prior to study participation. The study was performed in accordance with good clinical practices and the Declaration of Helsinki.

Clinical assessment

For each patient, we recorded clinical and demographic data (i.e., age, sex, disease duration [from disease onset]), DMTs, and descriptor of disease progression (progressive or relapsing) [14]. Each patient underwent a clinical examination, including assessment of physical disability through the Expanded Disability Status Scale (EDSS) [15].

Neuropsychometric assessment

Each patient underwent cognitive and psychometric evaluations. Cognitive function was assessed using the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) [16] battery, exploring attention, information-processing speed, working memory, verbal memory, and visual memory.

Specifically, attention, information-processing speed, and working memory were evaluated using the Symbol Digit Modalities Test (SDMT). Verbal and spatial memory were assessed using the California Verbal Learning Test–II (CVLT II) and the Brief Visuospatial Memory Test–Revised (BVMT-R). Scores were adjusted for age, gender, and education following Italian normative values [16]. Scores <35 were considered indicative of cognitive deficit. The total cerebral functional score (CFS) was calculated by summing the impaired cognitive domains. A CFS of 0 indicated normal scores across all cognitive domains, whereas scores ranging from 1 to 3 indicated deficits on one to three cognitive tests.

Additionally, each participant completed the Beck Depression Inventory (BDI) [17] to assess depressive symptoms and the modified Fatigue Impact Scale (MFIS) [18] to measure cognitive, physical, and psychosocial fatigue. Further assessments included the Beck Anxiety Inventory (BAI) [19] and the Pittsburgh Sleep Quality Index (PSQI) [20] to evaluate anxiety symptoms and sleep disorders.

Blood sample assessment

Each MS patients underwent blood draws on the same day of clinical and neuropsychological assessments. An aliquot (50 μL) of anticoagulated ethylenediaminetetraacetic acid whole fresh blood (within 12 h) was incubated at 4°C for 30 min in the presence of appropriate amounts of monoclonal antibodies. The mixtures were then diluted 1:20 in ammonium chloride lysing solution to remove erythrocytes (Pharmlyse; BD Bioscience), incubated at room temperature for 10 min and finally washed. Samples were analyzed on Becton Dickinson Facs Canto II cytometer BD Facs Diva software. The lower level of detection was 10-4 (as such, zero corresponds to a level <1/10,000 cells). The values have been expressed as a percentage.

Doublets and debris were excluded upon analysis and leukocytes gate. For lymphocyte absolute count, we coupled cytometry to complete blood count on hematological counter (double platform).

The following antigens were analyzed: CD3 Pacific Blu (Beckman Coulter) and CD20+ FITC (Beckman Coulter). The lower level of detection was 10-4 (as such, zero corresponds to a level <1/10,000 cells). The gating strategy was as follows. Lymphocyte cells were gated by using CD45 versus side scatter-A, identifying 50,000 events. This gate was used to identify T lymphocytes CD3+, B lymphocytes CD19+, and CD20+ and CD3+CD20dim. Laboratory procedures were performed in accordance with UK-NEQAS quality standards in the leucocyte immunophenotyping program (https://ukneqas.org.uk/). Patients were classified as positive for CD20 T lymphocytes if CD20 T lymphocytes were ≥0.2%.

Statistical analysis

Statistical analyses were performed using Stata/MP software (version 15.03, StataCorp). Demographic, clinical, and neuropsychological features of study subjects are presented as mean, median, or proportion as appropriate. All demographic, clinical, neuropsychological, and laboratory variables were checked for normality using the Shapiro–Wilk normality test.

Between-group (CD20+ T lymphocyte present or absent) comparisons of demographic and clinical features were performed using Student *t*-test, Wilcoxon rank-sum test, and chi-squared test tests as appropriate. Correlation between CD20+ T lymphocyte level and age, disease duration, and EDSS was assessed through pairwise Pearson correlation using post hoc Bonferroni correction. To evaluate associations between CD20+ T lymphocyte status and neuropsychological scores, we performed for each score a stepwise regression model with backward selection using $p=0.10$ as the critical value for removal of variables from the model, including age, sex, treatment-naive status, EDSS, disease course, and CD20+ T lymphocyte status (yes/no) as independent variables and CFS, corrected SDMT, corrected CVLT II, corrected BVMT-R, total, cognitive, physical, and psychosocial MFIS, BDI, BAI, and PSQI as dependent variables. Even if corrected cognitive scores already accounted for age and sex, we included these variables in the model to obtain the net coefficient of correlation between CD20+ T lymphocyte status (yes/no) and cognitive score. Normality distribution of residuals was assessed through Shapiro–Wilk test, and model residuals were visually inspected to ensure model homoscedasticity. A *p*-value <0.05 was be considered statistically significant. Results are presented with 95% confidence interval (95% CI) or *p*-value.

RESULTS

Clinical and laboratory measures at baseline

We enrolled 90 MS patients. We included 61 female (68%) and 29 male (32%) patients, with a mean age of 48.1 ± 12.7 years, a median disease duration of 12.4 (range =0–53) years, and a median EDSS of 4.0 (range = 1.0–7.5). Forty-four patients were relapsing–remitting (49%) and 46 patients were progressive (51%), with 43 patients having secondary progressive MS and three patients having primary progressive MS. Demographic and clinical data from subjects enrolled in the study are summarized in Table 1 and Table 2. Cognitive and neuropsychological features are depicted in Table 3.

Mean lymphocyte level was 18.1 ± 10.6% of circulating white blood cells, with mean CD20+ T lymphocytes being 0.38 ± 1.2% (median = 0, range = 0–6). Seventeen patients (18.9%) were classified as being positive for CD20+ T lymphocytes. Patient with CD20+ T lymphocytes were older (46.7 ± 13.2 vs. 54.3 ± 8.3 years, $p=0.03$), were more likely to be at progressive disease phases (76.5% of progressive patients were positive for CD20+ T lymphocytes vs. 23.5% of relapsing MS patients, $p=0.02$), and showed a higher EDSS (median [range] =6.0 [1.5–7.5] vs. 3.5 [1–7.5], $p=0.001$). DMT naive status and sex were not different according to CD20+ T lymphocyte presence. Similarly, among progressive MS patients, time from conversion was not different according to CD20+ T lymphocyte presence. Among the 17 patients positive for CD20+ T lymphocytes, three were undergoing anti-CD20

TABLE 1 Demographic and clinical features of patients with multiple sclerosis.

Characteristic	Value
Subjects, *N*	**90**
Sex, *n* (%)	
Male	29 (32)
Female	61 (68)
Age, years, mean (SD)	48.1 (12.7)
Education, years, mean (SD)	12.9 (3.7)
EDSS, median (range)	4 (1–7.5)
Disease duration, years, median (range)	16.7 (0–52.8)
Descriptor of disease course, *n* (%)	
Relapsing–remitting	44 (49)
Secondary progressive	43 (48)
Primary progressive	3 (3)
Time from conversion to secondary progressive phenotype, years, mean (SD)	7.2 (18.6)
DMT, *n* (%)	
No DMT	21 (23.3)
BRACE	5 (5.6)
Teriflunomide	1 (1.1)
Fumarate	4 (4.4)
S1P inhibitors	43 (47.8)
Azathioprine	1 (1.1)
Cladribine	2 (2.2)
Natalizumab	6 (6.7)
Anti-CD20	6 (6.7)
Anti-CD52	1 (1.1)

Abbreviations: BRACE, Betaferon, Rebif, Avonex, Copaxone, Extavia; DMT, disease-modifying treatment; EDSS, Expanded Disability Status Scale; S1P, sphingosine 1-phosphate.

TABLE 2 Demographic and clinical features of patients with MS according to descriptor of disease course.

Characteristic	Relapsing MS	Progressive MS	*p*
Subjects, *n*	44	46	
Sex, *n* (%)			
Male	9 (20)	20 (43)	0.02
Female	35 (80)	26 (57)	
Age, years, mean (SD)	42.7 (13.7)	53.3 (9.2)	<0.001
EDSS, median (range)	2.5 (1–7.5)	5.5 (2.5–7.5)	<0.001
Disease duration, years, median (range)	6.25 (0–52.8)	22.2 (0–51)	<0.001
DMT, *n* (%)			
No DMT	13 (29)	8 (17)	0.03
BRACE	1 (2)	4 (9)	
Teriflunomide	1 (2)	-	
Fumarate	2 (5)	2 (4)	
S1P inhibitors	17 (39)	26 (57)	
Azathioprine	-	1 (2)	
Cladribine	2 (5)	-	
Natalizumab	6 (14)	-	
Anti-CD20	1 (2)	5 (11)	
Anti-CD52	1 (2)	-	

Abbreviations: BRACE, Betaferon, Rebif, Avonex, Copaxone, Extavia; DMT, disease-modifying treatment; EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; S1P, sphingosine 1-phosphate.

drugs (previously on interferon treatment), one patient was undergoing azathioprine treatment (first-line treatment), two patients were treated with interferon (first-line treatment), seven patients were treated with an anti-sphingosine 1-phosphate drug (three previously treated with interferon, two previously treated with fumarate, and two previously treated with teriflunomide), one patient was undergoing fumarate treatment (first-line treatment), and three patients were naive to DMT. There was no difference in DMT between $CD20^+$ and CD^- T lymphocytes patients.

At stepwise backward selection, patients with $CD20^+$ T lymphocytes showed higher CFS score (coefficient = 0.75, 95% CI = 0.25–1.26, $p = 0.004$; Figure 1a), higher MFIS total score (coefficient = 13.41, 95% CI = 1.85–25, $p = 0.02$; Figure 1b), higher cognitive MFIS score (coefficient = 6.65, 95% CI = 1.47–11.83, $p = 0.01$; Figure 1d), higher psychosocial MFIS score (coefficient = 2.79, 95% CI = 0.86–4.72, $p = 0.005$; Figure 1e), and a trend toward worse sleep quality assessed through the PSQI (coefficient = 3.54, 95% CI = −0.08 to 7.15, $p = 0.06$; Figure 1h). Physical MFIS score, BAI, and BDI were not associated to $CD20^+$ T lymphocyte status (Figure 1c,f,g). Finally, in those patients positive for $CD20^+$ T lymphocytes, the level of $CD20^+$ T lymphocyte was not associated with each single BICAMS test. Detailed information about regression models unveiling significant association between $CD20^+$ T lymphocyte status and clinical features is reported in Table 4.

DISCUSSION

Our study investigated peripheral $CD20^+$ T lymphocytes in MS patients and their correlations with clinical and neuropsychological features. In our sample, 17 patients (18.9%) displayed $CD20^+$ T lymphocytes in blood samples. Patients showing $CD20^+$ T lymphocytes were older and had progressive disease course. Additionally, patients with $CD20^+$ T lymphocytes showed higher levels of fatigue, particularly cognitive and psychosocial fatigue.

Previous studies showed conflicting data regarding the presence of $CD20^+$ T lymphocytes in the peripheral blood of MS patients

It is difficult to compare our data on $CD20^+$ T lymphocyte prevalence in MS with those of other studies because they did not

report the prevalence of patients positive for CD20$^+$ T lymphocytes but an aggregated mean percentage of the CD20$^+$ T lymphocyte percentage.

TABLE 3 Age-, sex-, and education-adjusted cognitive scores and psychological features in multiple sclerosis patients.

Outcome	Score
SDMT, mean (SD)	42.1 (11.9)
CVLT, mean (SD)	40.6 (14.1)
BVMT, mean (SD)	43.7 (12.9)
CFS score, *n* (%)	
0	47 (52.2)
1	21 (23.3)
2	13 (14.5)
3	9 (10)
BDI-II, mean (SD)	11.7 (11.8)
MFIS cognitive, mean (SD)	8.5 (10)
MFIS physical, mean (SD)	12.1 (11.1)
MFIS psychosocial, mean (SD)	2.6 (3.8)
MFIS total, mean (SD)	22.8 (22)
PSQI, mean (SD)[a]	6.3 (6.6)
BAI, mean (SD)[b]	9 (11.1)

[a]For the PSQI, we collected data for 75 patients.

[b]For the BAI, we collected data for 71 patients.

Abbreviations: BAI, Beck Anxiety Inventory; BDI-II, Beck Depression Inventory; BVMT, Brief Visuo-spatial Memory Test; CFS, cerebral functional score. CVLT, California Verbal Learning Test; MFIS, Modified Fatigue Impact Scale; PSQI, Pittsburgh Sleep Quality Index; SDMT, Symbol Digit Modalities Test.

However, the assessment of percentage of CD20$^+$ T lymphocytes in other autoimmune disorders such as rheumatoid arthritis and psoriasis did not report on an increased prevalence of this cell population compared to healthy controls [21, 22].

Accordingly, we may hypothesize that CD20$^+$ T lymphocytes may underpin pathological processes not specifically involved in the systemic inflammatory cascade, but in compartmentalized inflammatory processes of CNS.

Previous studies assessing CD20$^+$ T lymphocytes in MS revealed a significantly higher proportion of CD20$^+$ T lymphocytes in the blood of patients with both relapsing–remitting MS and primary progressive MS compared to healthy controls, representing approximately 5%–15% of the CD4$^+$ and CD8$^+$ T cell compartments [5]. In particular, authors reported on higher proportions of CD8$^+$CD20$^+$ T lymphocytes in primary progressive MS versus relapsing–remitting MS, whereas proportions of CD4$^+$CD20$^+$ T lymphocytes were similar between progressive and relapsing MS. [5].

Therefore we may speculate that CD20$^+$ T lymphocytes, specifically in its CD8 counterpart, may play a role at progressive disease stages in MS. Future studies differentiating CD4 and CD8 CD20$^+$ T lymphocytes and their correlation with markers of MS pathology, namely, brain atrophy or cortical lesions, are required to confirm this hypothesis.

Although mechanisms underpinning the relationship between CD20$^+$ T lymphocytes and clinical progression are still debated, previous studies highlighted correlation of higher levels of CD20$^+$ T lymphocytes with disease severity. In a previous report, there was a correlation between the presence of CD20$^+$ T lymphocytes in the cerebrospinal fluid (CSF) of MS patients and EDSS score [5]. In addition, the proportion of CD20$^+$ T lymphocytes in the CSF of primary

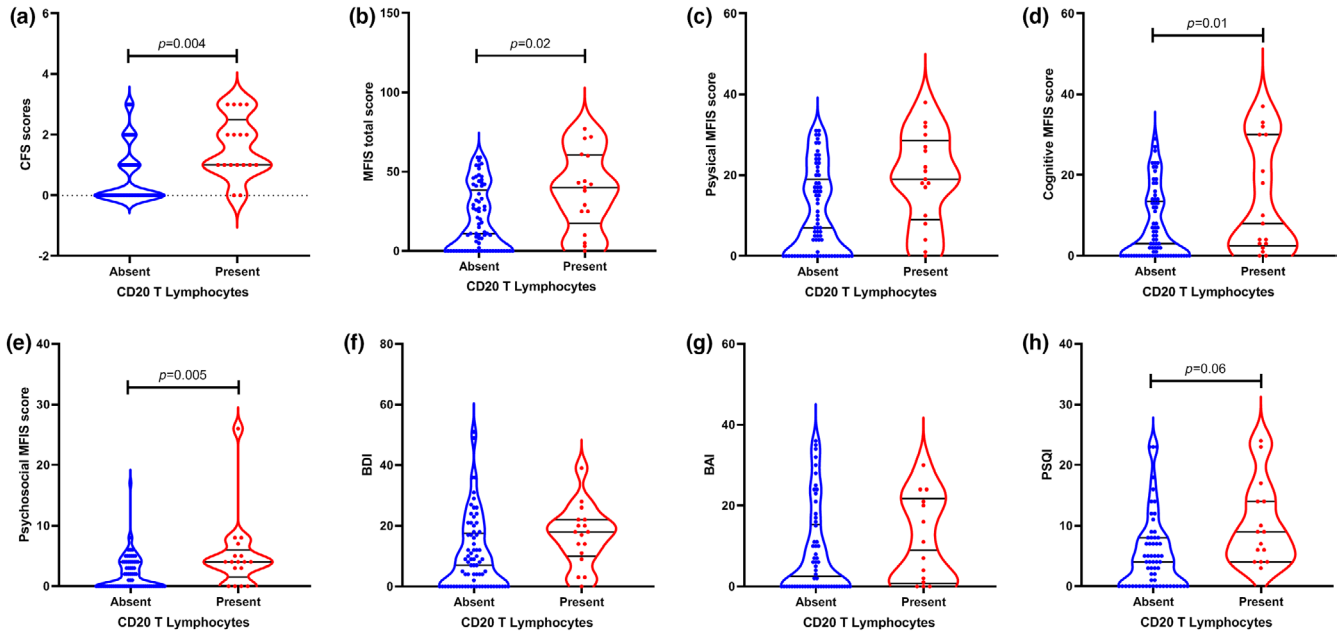

FIGURE 1 Association between neuropsychological tests and CD20$^+$ T lymphocyte status. Violin plots represent cerebral Brief International Cognitive Assessment for Multiple Sclerosis (a), modified Fatigue Impact Scale (MFIS) total (b), MFIS physical (c), MFIS cognitive (d), MFIS psychosocial (e), Beck Depression Inventory (BDI; f), Beck Anxiety Inventory (BAI; g), and Pittsburgh Sleep Quality Index (PSQI; h) scores according to CD20$^+$ T lymphocyte status. CFS, cerebral functional score.

TABLE 4 Regression models unveiling significant association between $CD20^+$ T lymphocyte status and clinical multiple sclerosis features.

Outcome	Predictor	Coefficient	95% CI, lower	95% CI, upper	*p*	R^2/R^2, adjusted
CFS						0.22/0.20
	Disease course					
	Relapsing	Ref.				
	Progressive	0.62	0.22	1.01	0.002	
	$CD20^+$ T lymphocyte status					
	Negative	Ref.				
	Positive	0.75	0.25	1.26	0.004	
MFIS total score						0.17/0.15
	EDSS	3.13	0.67	5.58	0.13	
	$CD20^+$ T lymphocyte status					
	Negative	Ref.				
	Positive	13.41	1.85	24.98	0.02	
Cognitive MFIS						0.07/0.06
	$CD20^+$ T lymphocyte status					
	Negative	Ref.				
	Positive	6.65	1.47	11.83	0.01	
Psychosocial MFIS						0.13/0.11
	Sex					
	Male	Ref.				
	Female	−1.54	−3.16	0.07	0.06	
	$CD20^+$ T lymphocyte status					
	Negative	Ref.				
	Positive	2.79	0.86	4.72	0.005	
PSQI						0.23/0.21
	EDSS	1.29	0.54	2.04	0.001	
	$CD20^+$ T lymphocyte status					
	Negative	Ref.				
	Positive	3.54	−0.08	7.16	0.06	

Abbreviations: CFS, cerebral functional score; CI, confidence interval; EDSS, Expanded Disability Status Scale; MFIS, Modified Fatigue Impact Scale; PSQI, Pittsburgh Sleep Quality Index; Ref., referent.

progressive MS patients was associated with markers of progressive pathological changes at progressive disease stages (i.e., myelin basic protein levels in the CSF and the total volume of white matter lesions) [23]. In our study, we highlighted an association between the presence of $CD20^+$ T lymphocytes and the extent of physical disability as assessed through the EDSS, therefore adding further confirmatory data on this association.

The main finding of our study was a higher expression of fatigue in patients positive for $CD20^+$ T lymphocytes in blood samples. Over recent years, several studies have investigated the pathogenesis of MS-related fatigue, attributing it to biological and psychological factors, including inflammation, demyelination, altered brain activation patterns, and functional abnormalities measured by functional MRI, as well as with depression and anxiety [24].

Brain atrophy is one of the well-established mechanisms associated with fatigue in MS, with morphometric studies reporting on a potential link between fatigue and brain atrophy [8, 25].

Compartmentalized immune responses in the CSF-filled compartment of the brain also greatly contribute further to the development of fatigue in MS patients [12].

Our study demonstrated the presence of $CD20^+$ T lymphocytes in MS patients exhibiting higher cognitive and psychosocial fatigue. Given the interplay of $CD20^+$ T lymphocytes and the clinical symptoms possibly associated with disease progression such as fatigue, which is in turn associated with compartmentalized inflammation [12], we may speculate that $CD20^+$ T lymphocytes may contribute to the pathogenetic mechanisms of fatigue by fueling mechanisms associated with compartmentalized inflammation. Future studies are required to specifically look at this association by applying proper neuroimaging technique (i.e., atrophy assessed through MRI for the neurodegenerative processes and translocator protein positron emission tomography as a marker of compartmentalized inflammation) [26]. In addition, it is relevant to note that fatigue has been previously associated with disability and descriptors of disease course

[27, 28]. Therefore, although we included in the stepwise analysis these two clinical features, we could not completely rule out that the association between fatigue and CD20 T lymphocytes could be due to the higher prevalence of progressive disease stages in the CD20$^+$ T lymphocyte population.

Our study has limitations. First, it is a cross-sectional monocentric study with a small sample size. This limitation could be addressed in future research by expanding the measurement of CD20$^+$ T lymphocytes to a larger population and collecting data from multiple centers. A larger sample would also allow a more granular analysis of the possible impact of the different DMTs and DMT sequencing on CD20 T lymphocytes.

Second, we did not collect CSF cells or MRI findings, which could further elucidate the role of CD20$^+$ T lymphocytes in MS pathogenesis. Additionally, we only assessed cognitive and psychosocial fatigue using a single scale, potentially limiting symptom assessment. Moreover, some patients were undergoing DMTs, and CD20$^+$ T lymphocyte measurements out of treatment were not obtained (nor can treatment-related changes be evaluated). In addition, the lack of a control group prevents us from drawing any conclusion about the specificity of the association between CD20$^+$ T lymphocytes and clinical symptoms in MS.

In conclusion, our study suggests that MS patients with circulating CD20$^+$ T lymphocytes are more likely to have clinical symptoms associated with progressive disease course. Peripheral CD20$^+$ T lymphocytes could potentially serve as markers for both disease progression and development of fatigue in MS patients.

AUTHOR CONTRIBUTIONS

Antonio Esposito: Methodology; writing – original draft; investigation; data curation. **Fabrizia Falco:** Data curation. **Giulia Scalia:** Data curation; formal analysis; methodology. **Laura Gentile:** Data curation; formal analysis. **Antonio Luca Spiezia:** Investigation; validation; visualization. **Giuseppe Corsini:** Investigation; validation; data curation. **Rosa Manganiello:** Formal analysis; data curation. **Martina Eliano:** Data curation. **Federica Lamagna:** Data curation. **Marcello Moccia:** Validation; supervision; writing – review and editing. **Maria Petracca:** Validation; writing – review and editing; supervision. **Roberta Lanzillo:** Validation; writing – review and editing; supervision. **Vincenzo Brescia Morra:** Validation; writing – review and editing; supervision. **Antonio Carotenuto:** Conceptualization; writing – review and editing; validation; methodology; supervision; data curation.

CONFLICT OF INTEREST STATEMENT

A.E. has received honoraria from Novartis. M.M. has received research grants from ECTRIMS-MAGNIMS, the UK MS Society, and Merck, and honoraria from Biogen, BMS Celgene, Ipsen, Janssen, Merck, Novartis, Roche, and Sanofi-Genzyme. A.C. has received research grants from Almirall, research grants from ECTRIMS-MAGNIMS, and honoraria from Almirall, Biogen, Roche, Sanofi-Genzyme, Merck, Ipsen, and Novartis. M.P. has received research grants from the Italian MS Foundation and Baroni Foundation, honoraria from Health & Life and Biogen, and sponsorship for travel/meeting expenses from Novartis, Roche, and Merck. R.L. has received honoraria from Biogen, Merck, Novartis, Roche, and Teva. V.B.M. has received research grants from the Italian MS Society and Roche, and honoraria from Bayer, Biogen, Merck, Mylan, Novartis, Roche, Sanofi-Genzyme, and Teva. None of the other authors has any conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The anonymized dataset used and analyzed during the current study is available from the corresponding author upon reasonable request.

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