IMMUNOSENESCENCE AND MULTIPLE SCLEROSIS: PROGNOSTIC AND THERAPEUTIC IMPLICATIONS

EDITED BY: Emanuele D'amico, Aurora Zanghì, Carlo Avolio and Hans-Peter Hartung

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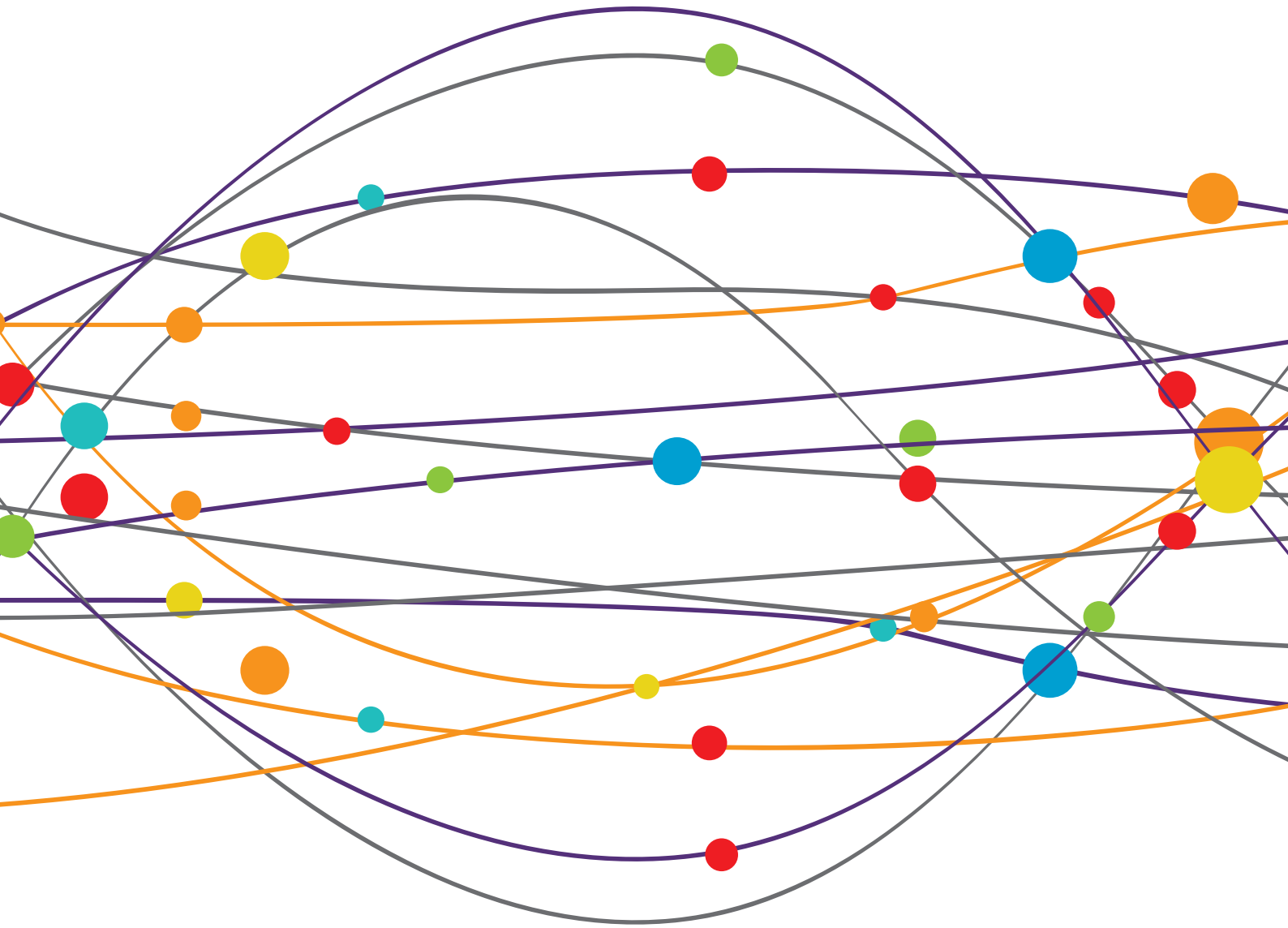

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IMMUNOSENESCENCE AND MULTIPLE SCLEROSIS: PROGNOSTIC AND THERAPEUTIC IMPLICATIONS

Topic Editors:
Emanuele D'amico, University of Catania, Italy
Aurora Zanghì, Sant'Elia Hospital, Italy
Carlo Avolio, University of Foggia, Italy
Hans-Peter Hartung, Heinrich Heine University of Düsseldorf, Germany

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Robert Weissert,
University of Regensburg, Germany

*CORRESPONDENCE
Emanuele D'Amico
emanuele.damico@unifg.it

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Editorial: Immunosenescence and multiple sclerosis: Prognostic and therapeutic implications

Aurora Zanghì[1], Carlo Avolio[2,3], Hans-Peter Hartung[4,5,6] and Emanuele D'Amico[2]*

[1]Unitá Operativa Complessa (UOC) Neurology, Sant'Elia Hospital, Caltanissetta, Italy, [2]Department of Medical and Surgical Sciences, University of Foggia, Foggia, Italy, [3]Department of Neurosciences, Head of Multiple Sclerosis Center, Policlinico Riuniti Hospital, Foggia, Italy, [4]Department of Neurology, Medical Faculty, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany, [5]Brain and Mind Center, The University of Sydney, Sydney, NSW, Australia, [6]Department of Neurology, Palacky University Olomouc, Olomouc, Czechia

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Editorial on the Research Topic
Immunosenescence and multiple sclerosis: Prognostic and therapeutic implications

Multiple sclerosis (MS) is a multifaceted disorder that mainly affects young adults, and it dramatically impacts their work and social abilities. The so-called late-onset MS (LOMS) is still considered very rare with a prevalence ranging from 4 to 9.4% according to different cohort studies (1, 2). LOMS displays several differences compared to young onset MS in terms of clinical and paraclinical characteristics, and it seems that LOMS more frequently presents a progressive course from the onset with a shorter time to severe motor disability; this could be associated with the higher incidence of comorbidities and polypharmacy (3, 4). As MS is a prototypic autoimmune disease of the central nervous system, we have to take into account how the immune system presents profound changes, both quantitatively and functionally, during an individual's lifetime, and it is important to understand the differences between these patients and younger patients.

Immunosenescence is defined as age-related changes in the immune system, leading to an increase in morbidity and mortality in older adults (5). The most important processes of immunosenescence are associated with a decrease in the number of naive T and B cells, NK cells, and disruption of the pro- and anti-inflammatory balance by changes in the production of cytokines (5).

The approval of a new disease modifying treatment (DMTs) for MS and the aging of population makes it increasingly important that we attempt to explain how the immune system alterations can be additive to, or work in synergy with, the changes induced by DMTs concerning the risk of infections and the disease course (activity and progression).

The aim of this Research Topic has been to provide new insights into the immunosenescence phenomenon in the MS population to identify how aging can influence disease trajectory and response/tolerability to DMTs (6).

The Authors have contributed with 12 valuable works on different aspects of immunosenescence in MS, including two reviews and two mini-reviews.

In particular, Li et al. analyzed and compare different clinical, laboratory, and magnetic resonance imaging characteristics between pediatric and adult patients with first-attack myelin oligodendrocyte glycoprotein antibody disease (MOGAD) to explore predictive factors for severity at disease onset. The authors found the clinical phenotype of MOGAD varies in patients of different ages (Li et al.).

Focusing on T-cell senescence, Tomas-Ojer et al. investigated the involvement of antigen-induced T-cell senescence in controlling CD4+ T-cell-mediated autoimmune responses in MS. Here, patients with high levels of CD4+ T-cell senescence in peripheral blood showed increased frequencies of CSF-infiltrating CD28+ CD27-EM CD4+ T cells with a proinflammatory Th1 functional phenotype. The correlation of these cells with the intrathecal levels of neurofilament light chain, a marker of neurodegeneration, suggests their relevance in disease pathogenesis and the involvement of T-cell senescence in their regulation. Markers of antigen-induced T-senescence, therefore, could promise as a tool to identify pathogenic CD4+ T cells in patients with MS (Tomas-Ojer et al.).

Perdaens and van Pesch focused on consequences of age-related immune changes on MS pathology in terms of interaction with the intrinsic aging process of central nervous system resident cells, and then they discussed the impact of immunosenescence on disease evolution and the safety and efficacy of current DMTs.

Furthermore, Ysrraelit and Correale discussed the role of androgens in the development and function of the innate and adaptive immune response as well as in neuroprotective mechanisms relevant to MS; evidence of epidemiological studies has shown a later age of onset of MS in men, relative to women, which could perhaps correspond to the decline in protective testosterone levels (Ysrraelit and Correale).

Manouchehri et al. discussed the role of immune senescence on different arms of the immune system and how it may explain relative DMT resistance based on the classical dichotomy of DMT effectiveness between relapsing MS and progressive MS, which is informative of distinct pathogeneses of the different MS phenotypes (7).

The use of DMT in the elderly population has been investigated by Ng et al.. They conducted a population-based observational study using linked administrative health data from British Columbia, Canada. Their results showed that any DMT, vs. no DMT, in the <55-year-olds was associated with a 23% lower hazard of hospitalization (adjusted hazard ratio, aHR 0.77; 95% CI 0.72–0.82), but not in the ≥55-year-olds (aHR 0.95; 95% CI 0.87–1.04) (Ng et al.).

Buscarinu et al. discussed how the aging process influences the onset, the clinical course, and the therapeutic approach in LOMS.

Giovannoni et al. performed an Integrated Lymphopenia Analysis in Younger and Older patients treated with Cladribine (Clad) at a dosage of 3.5 mg/kg, focusing on the possible effect of Clad on lymphocyte levels by age. Overall, lymphocyte recovery began soon after nadir following Clad treatment and median levels reached normal range by end of the treatment year in both age groups. The rate of certain infections was numerically higher in older vs. younger patients (Giovannoni et al.). In conclusion, Clad had a similar effect on ALC and lymphocyte subsets in both younger and older patient groups (Giovannoni et al.).

Vollmer et al. in a real-world cohort of relapsing MS patients, revealed high-efficacy DMTs had less benefit with aging but were associated with increased risks. These results help overcome some limitations of trials where older patients were excluded. To better balance benefits/risks, authors have proposed a DMT de-escalation approach for aging MS patients (Vollmer et al.).

Real-world data and post-marketing surveillance are certainly of interest since many patients who started DMT over the last decades are currently older than the age limits usually used in clinical trials. Wandall-Holm et al. proposed the results from a Danish MS cohort and revealed patients with MS are at a higher risk of losing all income from earnings and at a much higher risk of receiving disability pension compared with healthy controls.

This special issue warrants further study specifically designed to quantify risk and to disclose better strategies to minimize such a risk.

Author contributions

AZ: conceptualization, methodology, project administration, and writing—original draft preparation. CA and H-PH: conceptualization and writing—original draft preparation. ED'A: conceptualization, methodology, writing—original draft preparation, writing—review and editing, supervision, and final validation. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

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Monocyte to High-Density Lipoprotein Ratio: A Novel Predictive Marker of Disease Severity and Prognosis in Patients With Neuromyelitis Optica Spectrum Disorders

*Jinwei Zhang†, Yanfei Li†, Yongyan Zhou, Kaixin Wang, Chunyang Pan, Yi Zhao, Haojie Xie, Ranran Duan, Zhe Gong and Yanjie Jia**

Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

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Hans-Peter Hartung, Heinrich Heine University of Düsseldorf, Germany

Reviewed by:
Emanuele D'amico, University of Catania, Italy
Shuhei Nishiyama, Massachusetts General Hospital and Harvard Medical School, United States

***Correspondence:**
Yanjie Jia
jiayanjie1971@zzu.edu.cn

†These authors have contributed equally to this work and share first authorship

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Background and Purpose: To investigate the association of monocyte to high-density lipoprotein ratio (MHR) with disease severity and prognosis in patients with neuromyelitis optica spectrum disorders (NMOSD).

Methods: This retrospective study included 125 patients with NMOSD. Demographic and clinical parameters, including the MHR, were assessed. The initial Expanded Disability Status Scale (EDSS) score and relapse rate were used to evaluate disease severity and prognosis, respectively. Correlations between MHR and disease severity and relapse rate were analyzed. The predictive value of MHR for prognosis was evaluated using receiver operating characteristic (ROC) curve analysis.

Results: Compared with the low MHR group, the initial EDSS score (median 4.5 vs. 5.5%, $P = 0.025$) and relapse rate (51.61 vs. 30.16%, $P = 0.015$) were significantly higher in the high MHR group. MHR was positively correlated with the initial EDSS score ($r = 0.306$, $P = 0.001$). Multivariate analysis showed that MHR was significantly associated with severity (odds ratio = 7.90, 95% confidence interval [CI] = 1.08–57.82, $P = 0.041$), and it was a significant predictor of disease prognosis (hazard ratio = 3.12, 95% CI = 1.02–9.53, $P = 0.046$). The median relapse interval of the high MHR group was 24.40 months. When the MHR was higher than 0.565, the risk of relapse was high [sensitivity, 33.3%; specificity, 91.9%; area under the ROC curve, 0.642 (95% CI = 0.54–0.74, $P = 0.007$)].

Conclusion: MHR is a novel predictive marker of disease severity and prognosis in patients with NMOSD. Early monitoring and reduction of MHR may allow earlier intervention and improved prognosis.

Keywords: neuromyelitis optica spectrum disorders, monocyte to high-density lipoprotein ratio, prognosis, severity, expanded disability status scale

INTRODUCTION

Neuromyelitis optica is an autoimmune demyelinating disease of the central nervous system that is characterized by acute optic neuritis and transverse myelitis occurring simultaneously or continuously (1–3), with an estimated prevalence of 1–2 per 100,000 people. Approximately 80% of patients have specific antibodies to astrocyte aquaporin 4 (AQP4), and this is one of the key diagnostic criteria (4–6). Patients with neuromyelitis optica spectrum disorders (NMOSD) have severe immune-mediated attacks that usually lead to severe residual disability and a high relapse rate (7). Therefore, accurate prediction of relapse is important to help clinicians initiate early preventive treatment and improve patient prognosis (8, 9).

As an important effector cell of the innate immune response, monocytes play a key role in the pathogenesis of autoimmune-related central nervous system diseases, including NMOSD (10–13). Studies have shown that anti-AQP4 antibodies can stimulate astrocytes to release chemokines, recruit monocytes and promote their activation, enhance the natural immune response, and destroy the blood-brain barrier, which plays a key role in accelerating the formation of NMOSD lesions (14–17). High-density lipoprotein (HDL) is considered an anti-inflammatory factor that has immunomodulatory and antioxidant effects on endothelial cells (18–20) and can prevent the production of pro-inflammatory cytokines. Studies have shown that low HDL is related to disease activity and disability in patients with AQP4-positive NMOSD, which may be associated with the decrease in the levels of apolipoprotein (apo) A-I, the main component of HDL in serum (21, 22).

The monocyte to high-density lipoprotein ratio (MHR) is a novel marker that reflects the degree of inflammation and oxidative stress. Many studies have shown that the MHR is closely related to the occurrence, development, and prognosis of cardiovascular, cerebrovascular (23–25), immune system (26), and rheumatic diseases (27–30). For example, one study found that the MHR was significantly higher in patients with multiple sclerosis than in healthy controls and that it was related to disease severity and disability, suggesting that the MHR may be used as an independent index to predict disability (31). However, the correlation between MHR and prognosis and relapse in patients with NMOSD remains unclear. The purpose of this study was to explore the relationship between MHR and disease severity and prognosis in patients with NMOSD and to determine the best cutoff value of MHR to predict the prognosis of patients with NMOSD.

MATERIALS AND METHODS

Patients

This study was approved by the Ethics Committee of Zhengzhou University (2019-KY-018). All patients provided written informed consent to participate in this study. In this retrospective study, we collected the clinical data of 650 patients newly diagnosed with NMOSD from September 2013 to June 2020 at the First Affiliated Hospital of Zhengzhou University. The inclusion criteria were: (1) diagnosed with NMOSD for the first time according to the 2006 Wingerchuk standard or the 2015 McDonald NMOSD international general diagnostic standard (32, 33); (2) no serious liver or kidney damage, serious cardiovascular or cerebrovascular diseases, malignant tumors, blood system diseases, or other immune diseases; (3) no chronic systemic inflammatory diseases or history of recent infection; (4) did not receive treatment with anti-inflammatory drugs within 10 days before admission, no lipid-lowering treatment within 6 months before admission, and were not taking drugs that affect the number of white blood cells and blood lipid levels; (5) did not receive immunosuppressant treatment within 6 months before admission, and (6) had complete clinical data and follow-up data. A total of 125 patients met these criteria and were included in this cohort study. The specific screening process is shown in **Figure 1**.

Data Collection

Clinical data were obtained through a retrospective review of the hospital electronic case system. The following clinical information was collected: gender, age, clinical phenotype at onset, comorbidities, blood cell count, triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), HDL, MHR, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), anti-AQP4 antibody status, spinal magnetic resonance imaging (MRI), and treatment plan.

Blood samples were collected from the antecubital vein at 8:00 am after an overnight fast and stored on ice before testing (within 30 min after collection). Blood samples were analyzed in the biochemistry laboratory of the hospital. Blood cell count and all biochemical parameters were determined by standard methods, and the enzyme kit (Sigma-Aldrich, Saint Louis, MO, USA) was used to evaluate fasting TC, TG, LDL, and HDL levels. The anti-AQP4 antibody status in serum or cerebrospinal fluid samples was analyzed by the cell-based assay method. MRI of the spinal cord was performed using a 3T MAGNETOM Skyra scanner (Siemens Healthcare, Erlangen, Germany) in the Department of Magnetic Resonance of the hospital at admission. Longitudinally extensive transverse myelitis (LETM) was considered as extending 3 or more vertebral segments on T2-weighted imaging (33). When more than one test was performed during hospitalization, the data from the first test were included in the analysis. All tests were conducted according to the manufacturer's instructions, and the inspectors were blinded to the diagnosis or clinical symptoms.

Clinical Assessment

To calculate the extended disability status scale (EDSS) score at the time of admission, at least two professional neurologists carefully reviewed the patient's clinical records. This was recorded as the initial EDSS score, which was used to evaluate disease severity. The baseline EDSS scores of all patients before the first attack were considered normal. Patients in the cohort were divided into those with mild disability (EDSS score 0–3.5) or moderate/severe disability (EDSS score 4–9.5) (34–36).

The main index for evaluating prognosis was the relapse rate, which was defined as new or relapsed neurological symptoms caused by demyelinating diseases of the central nervous system

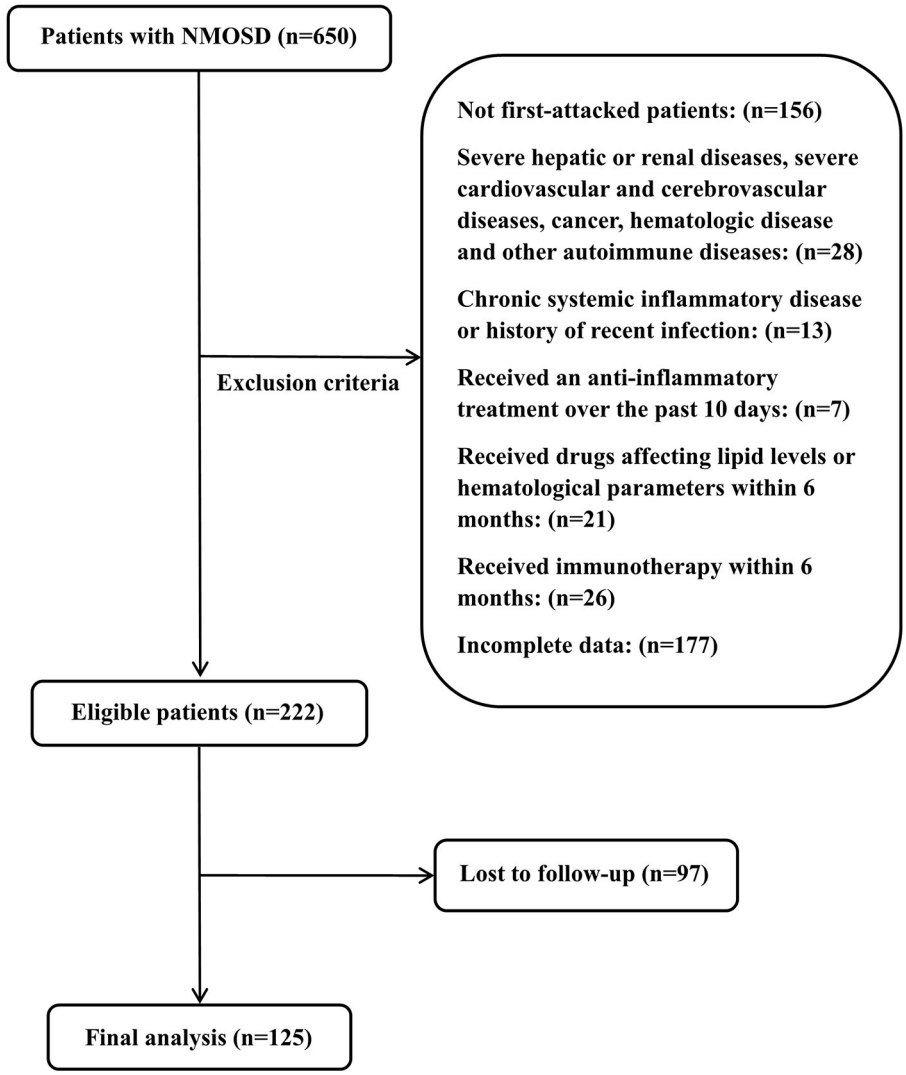

FIGURE 1 | Flow chart of the study population. NMOSD, neuromyelitis optica spectrum disorders.

without fever or infection, lasting at least 24 h, and increasing the existing EDSS score of patients by 0.5 points (37). Follow-up data were obtained by a clinical visit or telephone review every 6 months.

Statistical Analysis

Statistical analysis was performed using SPSS version 26.0 (International Business Machines Corporation, Chicago, Illinois, USA). According to the median MHR, the cohort was divided into two groups, and the classification data were expressed as percentages (%). The chi-square test or Fisher's exact test was used to compare the two groups. The Kolmogorov-Smirnov test was used for normality testing, and the measurement data conforming to normal distribution were expressed as mean and standard deviation; the independent sample *t*-test was used for comparison between the two groups. Data with non-normal distribution were expressed as median and interquartile range, and the Mann-Whitney *U* test was used for comparison between the two groups. The correlation between MHR and other clinical indices and the initial EDSS score was obtained by Spearman correlation analysis. Patients in the cohort were divided into those with mild disability (EDSS score 0–3.5) or moderate/severe disability (EDSS score 4–9.5) (34–36). We used a binary logistic regression model to analyze the correlation between MHR and other clinical-related indicators and disease severity. Variables with $P < 0.2$ in univariate analysis and those that were closely related to dependent variables in the clinic were included in the multivariate model, and the results were expressed with the odds ratio (OR) and 95% confidence interval (95% CI). The Kaplan-Meier curve was used to analyze whether different MHRs had an independent influence on the time of the first relapse, and univariate Cox survival analysis was used to screen the variables with $p < 0.2$. Then the multivariate regression model was used to analyze whether MHR was a predictor of relapse in patients with

NMOSD. The results were expressed as the risk ratio with the 95% CI. We used a receiver operating characteristic (ROC) curve to analyze the predictive value of MHR for disease prognosis, determine its optimal critical value, and calculate the area under the curve to evaluate the accuracy of the cutoff value. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Demographic and Clinical Characteristics of Participants

A total of 125 patients with NMOSD were enrolled in this cohort study (**Figure 1**). The average age at onset was 42.53 ± 15.06, 106 patients were women (84.80%), 75 were positive for anti-AQP4 antibody (60%), and the average follow-up time was 37.87 (20.27–51.85) months. The most common clinical phenotype at onset in the whole cohort was optic neuritis (72%), and 66.40% of patients had extensive transverse myelitis on MRI of the spinal cord. To better evaluate the relationship between different MHRs and disease severity and prognosis, we divided patients into MHR ≤ 0.40 and MHR > 0.40 groups, according to the median MHR. There were no significant differences in age, gender, clinical phenotype and hypertension between the two groups. Compared to the low MHR group, the high MHR group had significantly lower TC levels (3.93 vs. 4.44 mmol/L, $P = 0.005$), and there were significantly more patients with LETM on MRI of the spinal cord (47 vs. 36, $P = 0.027$). Other clinical

TABLE 1 | Demographic and clinical parameters of patients with NMOSD.

	Total (*n* = 125)	**MHR ≤ 0.40 (*n* = 63)**	**MHR > 0.40 (*n* = 62)**	***P***
Age at onset, years, mean ± SD	42.53 ± 15.06	43.51 ± 14.10	41.53 ± 16.04	0.527
Gender, female, n (%)	106 (84.80)	55 (87.30)	51 (82.26)	0.432
Clinical phenotype at onset, n (%)				
Optic neuritis	72 (57.60)	32 (50.79)	40 (64.52)	0.121
Acute myelitis	38 (30.40)	21 (33.33)	17 (27.42)	0.472
Optic neuritis+ acute myelitis	10 (8.00)	5 (7.94)	5 (8.06)	0.979
Other combinations	25 (20.00)	15 (23.81)	10 (16.13)	0.283
Hypertension, *n* (%)	10 (8.00)	5 (7.94)	5 (7.81)	0.979
Anti-AQP4 status, n (%)				
Positive	75 (60.00)	38 (60.32)	37 (59.68)	0.942
Negative	50 (40.00)	25 (39.68)	25 (40.32)	
Laboratory test results, median (IQR)				
Monocytes, $\times 10^9$/L	0.49 (0.34–0.63)	0.34 (0.25–0.46)	0.59 (0.52–0.79)	<0.001*
Lymphocytes, $\times 10^9$/L	1.50 (1.08–2.20)	1.38 (1.00–2.15)	1.59 (1.14–2.49)	0.133
TC, mmol/L	4.29 (3.51–5.01)	4.44 (3.90–5.23)	3.93 (3.24–4.88)	0.005*
TG, mmol/L	1.08 (0.72–1.59)	1.03 (0.68–1.72)	1.10 (0.76–1.50)	0.743
HDL, mmol/L	1.23 (1.01–1.51)	1.37 (1.15–1.70)	1.07 (0.90–1.34)	<0.001*
LDL, mmol/L	2.70 (1.96–3.32)	2.79 (2.07–3.41)	2.46 (1.95–3.26)	0.052
ESR, mm/h	10.00 (7.25–16.00)	12.00 (7.00–16.00)	9.60 (7.33–16.50)	0.628
CRP, mg/L	1.50 (0.41–3.55)	1.07 (0.33–3.28)	1.50 (0.67–4.54)	0.144
Therapy regimens, n (%)				
Corticosteroid	119 (95.20)	60 (95.24)	59 (95.16)	0.984
Immunosuppressant	49 (39.20)	20 (31.75)	29 (46.77)	0.085
Intravenous immunoglobulin	16 (12.80)	9 (14.29)	7 (11.29)	0.616
Rehabilitation	4(3.20)	2 (3.17)	2 (3.23)	1.000
Spinal cord MRI, n (%)				
LETM	83 (66.40)	36 (57.14)	47 (75.81)	0.027*
STM	42 (33.60)	27 (42.86)	15 (24.19)	
Initial EDSS, median (IQR)	5.00 (3.25–6.50)	4.50 (3.00–6.00)	5.50 (4.00–7.00)	0.025*
Relapse, *n* (%)	51 (40.80)	19 (30.16)	32 (51.61)	0.015*
Follow-up time, median (IQR)	37.87 (20.27–51.85)	39.13 (17.50–56.07)	37.45 (21.17–50.82)	0.680

Continuous variables were presented as mean ± SD or median (IQR = 25th−75th percentile), and categorical variables were described as percentages (%).
MHR, monocyte to high-density lipoprotein ratio; AQP4, aquaporin-4; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; MRI, magnetic resonance imaging; LETM, longitudinally extensive transverse myelitis; STM, short-segment transverse myelitis; EDSS, Expanded Disability Status Scale.
**P < 0.05.*

parameters, such as lymphocyte count, TG, LDL, ESR, and CRP, were not significantly different between the two groups. There was a significant difference in the initial EDSS score between the two groups (5.5 vs. 4.5, $P = 0.025$). The relapse rate of the high MHR group was higher than that of the low MHR group (51.61 vs. 30.16%, $P = 0.015$). Other demographic and clinical characteristics are shown in **Table 1**.

Correlations Between MHR and Disease Severity in Patients With NMOSD

Spearman correlation analysis showed that the levels of monocytes ($r = 0.210$, $P = 0.019$), HDL ($r = -0.236$, $P = 0.008$), and CRP ($r = 0.237$, $P = 0.008$) were significantly correlated with the initial EDSS score of patients with NMOSD, but the correlation was weak (**Figures 2A–C**). There was no obvious correlation between the blood lymphocyte count, ESR, blood lymphocyte to HDL ratio, and disease severity ($P > 0.05$) (**Table 2**). In addition, the MHR was positively correlated with the initial EDSS score ($r = 0.306$, $P = 0.001$) (**Figure 2D**).

Univariate logistic regression analysis showed that the MHR was significantly correlated with disease severity (OR = 9.55, 95% CI = 1.35–67.77, $P = 0.024$). HDL levels (OR = 0.49, 95% CI = 0.20–1.22, $P = 0.126$) and MRI of the spinal cord showing LETM (OR = 1.82, 95% CI = 0.82–4.03, $P = 0.141$) had a moderate influence on the initial EDSS score. However, age, gender, hypertension, anti-AQP4 antibody status, blood monocytes, lymphocyte count, TC, TG, LDL, ESR, CRP, and lymphocyte to HDL ratio were not significantly correlated with the initial EDSS score. In the multivariate model, MHR was an independent risk factor for disease severity (OR = 7.90, 95% CI = 1.08–57.82, $P = 0.041$) (**Table 3**).

TABLE 2 | Correlation analysis of clinical parameters and initial EDSS scores.

	r	*P*
Monocytes, $\times 10^9$/L	0.210	0.019*
Lymphocytes, $\times 10^9$/L	−0.118	0.189
HDL, mmol/L	−0.236	0.008*
ESR, mm/h	0.022	0.806
CRP, mg/L	0.237	0.008*
Lymphocytes/HDL	0.014	0.874
MHR	0.306	0.001*

EDSS, Expanded Disability Status Scale; HDL, high density lipoprotein; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; MHR, monocyte to high-density lipoprotein ratio.
**P < 0.05.*

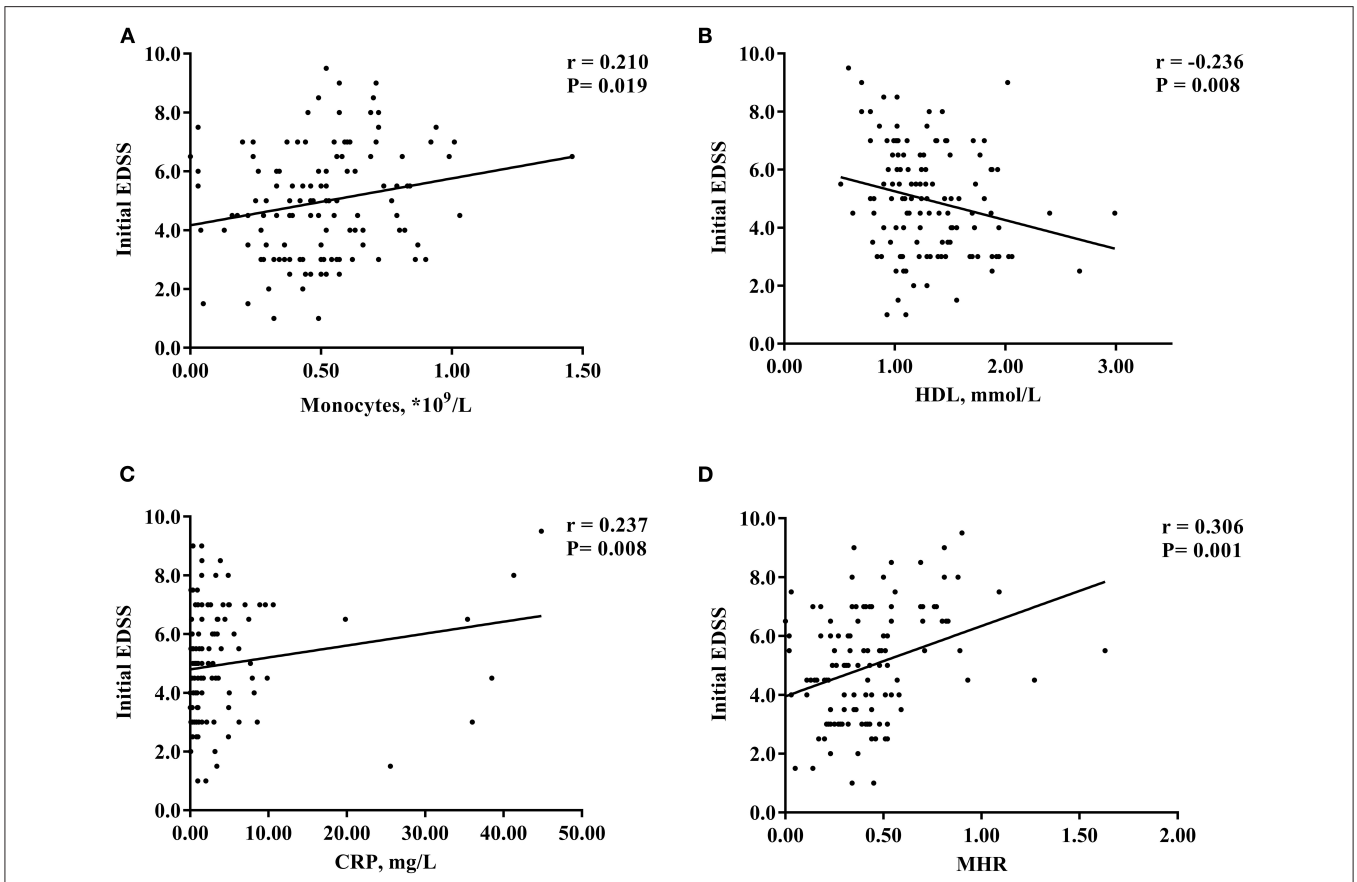

FIGURE 2 | Scatter plot of the correlation between monocytes **(A)**, HDL **(B)**, CRP **(C)**, MHR **(D)** and initial EDSS scores. HDL, high-density lipoprotein; MHR, monocyte to high-density lipoprotein ratio; CRP, C-reactive protein; EDSS, Expanded Disability Status Scale. *$P < 0.05$.

Correlations Between MHR and Prognosis in Patients With NMOSD

Kaplan-Meier analysis was used to evaluate the correlation between MHR and the relapse rate of patients with NMOSD.

TABLE 3 | Binary logistic regression analysis of disease severity in patients with NMOSD.

	Univariate analysis		Multivariable analysis	
	OR (95% CI)	**P**	**OR (95% CI)**	***P***
Age at onset	1.02 (0.99–1.04)	0.201		
Gender, female	1.12 (0.39–3.20)	0.837		
Hypertension	1.66 (0.44–6.25)	0.456		
Anti-AQP4 status, positive	1.03 (0.47–2.26)	0.936		
Monocytes	2.72 (0.49–14.95)	0.251		
Lymphocytes	1.02 (0.70–1.49)	0.922		
TC	0.98 (0.74–1.31)	0.913		
TG	1.05 (0.68–1.64)	0.814		
HDL	0.49 (0.20–1.22)	0.126		
LDL	1.03 (0.70–1.52)	0.876		
ESR	0.99 (0.97–1.02)	0.600		
CRP	1.02 (0.97–1.09)	0.416	1.02 (0.96–1.08)	0.559
Lymphocytes/HDL	1.27 (0.78–2.08)	0.333		
Monocytes/HDL (MHR)	9.55 (1.35–67.77)	0.024*	7.90 (1.08–57.82)	0.041*
Spinal cord MRI, LETM	1.82 (0.82–4.03)	0.141	1.66 (0.74–3.77)	0.222

OR, Odds ratio; CI, confidence interval; AQP4, aquaporin-4; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; MRI, magnetic resonance imaging; LETM, longitudinally extensive transverse myelitis; MHR, monocyte to high-density lipoprotein ratio.

**P < 0.05.*

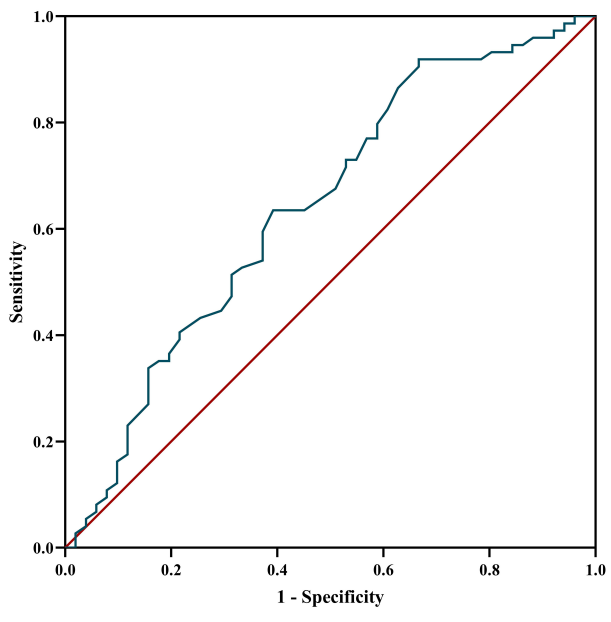

FIGURE 3 | Kaplan-Meier curves categorized according to MHR. MHR, monocyte to high-density lipoprotein ratio. **P* < 0.05.

MHR was a significant predictor of disease prognosis (log-rank P=0.046). The median relapse interval of the high MHR group was 24.40 months, whereas 50% of patients in the high MHR group relapsed 28.60 months after treatment (**Figure 3**).

Cox survival analysis was used to test the independent influence of MHRs on the relapse rate of patients with NMOSD. Univariate analysis showed that the MHR (HR = 3.01, 95% CI = 1.19–7.60, $p = 0.020$) was significantly correlated with relapse risk. However, there was no correlation between age, gender, hypertension, anti-AQP4 antibody, blood lymphocyte count, CRP, lymphocyte-to-HDL ratio, initial EDSS score, treatment plan, and relapse rate ($P > 0.05$). Blood mononuclear cell count, TC, TG, HDL, LDL, ESR, and MRI of the spinal cord showing LTEM had a moderate influence on disease relapse (**Figure 4A**). To eliminate these confounding factors, a multivariate Cox analysis model was established to obtain the corrected hazard ratio value. Because MHR is collinear with blood mononuclear cell count and HDL, these parameters were not included in the multivariate analysis. The results showed that the above confounding factors had no significant influence on the experimental results and that MHR was an independent risk factor for disease relapse (HR = 3.12, 95% CI = 1.02–9.53, $P = 0.046$) (**Figure 4B**).

The ROC curve was used to analyze the predictive value of MHR for disease prognosis. The area under the curve was 0.642 (95% CI = 0.54–0.74, $P = 0.007$), the best cutoff value was MHR = 0.565, the sensitivity was 0.333, and the specificity was 0.919, showing a good predictive ability for disease relapse. When the MHR is higher than 0.565, the risk of relapse is high (**Figure 5**).

DISCUSSION

Previous studies have demonstrated the prognostic value of the MHR in immune-mediated diseases, and in this study, we evaluated the effect of the MHR on the disease severity and prognosis of patients with NMOSD. We found that high MHR was associated with more severe disease at onset and a higher relapse rate, and the MHR at the onset of NMOSD was positively correlated with the initial EDSS score. Further, MHR was found to be an independent predictor of the severity and prognosis of NMOSD, and the best cutoff value to predict prognosis was 0.565.

As important effector cells of the innate immune response, monocytes have many functions, including antigen presentation, phagocytosis, and cytokine production, and their role in autoimmune diseases has attracted increasing attention (10–13). The blood mononuclear cell count is closely related to the early clinical severity of MS and can be used as a prognostic index (11). In our study, the blood mononuclear cell count was positively correlated with the NMOSD initial EDSS score. Studies have shown that in NMOSD, anti-AQP4 antibodies can stimulate astrocytes to release chemokines (14) and enhance monocyte recruitment and activation. Activated monocytes promote the production of inflammatory cytokines, such as tumor necrosis factor α, interleukin (IL)-6, IL-1β, IL-12, and IL-23 (38), and increase the expression of costimulatory molecules, such as CD80, ICAM-1, and HLA-DR (39), whereas the level of

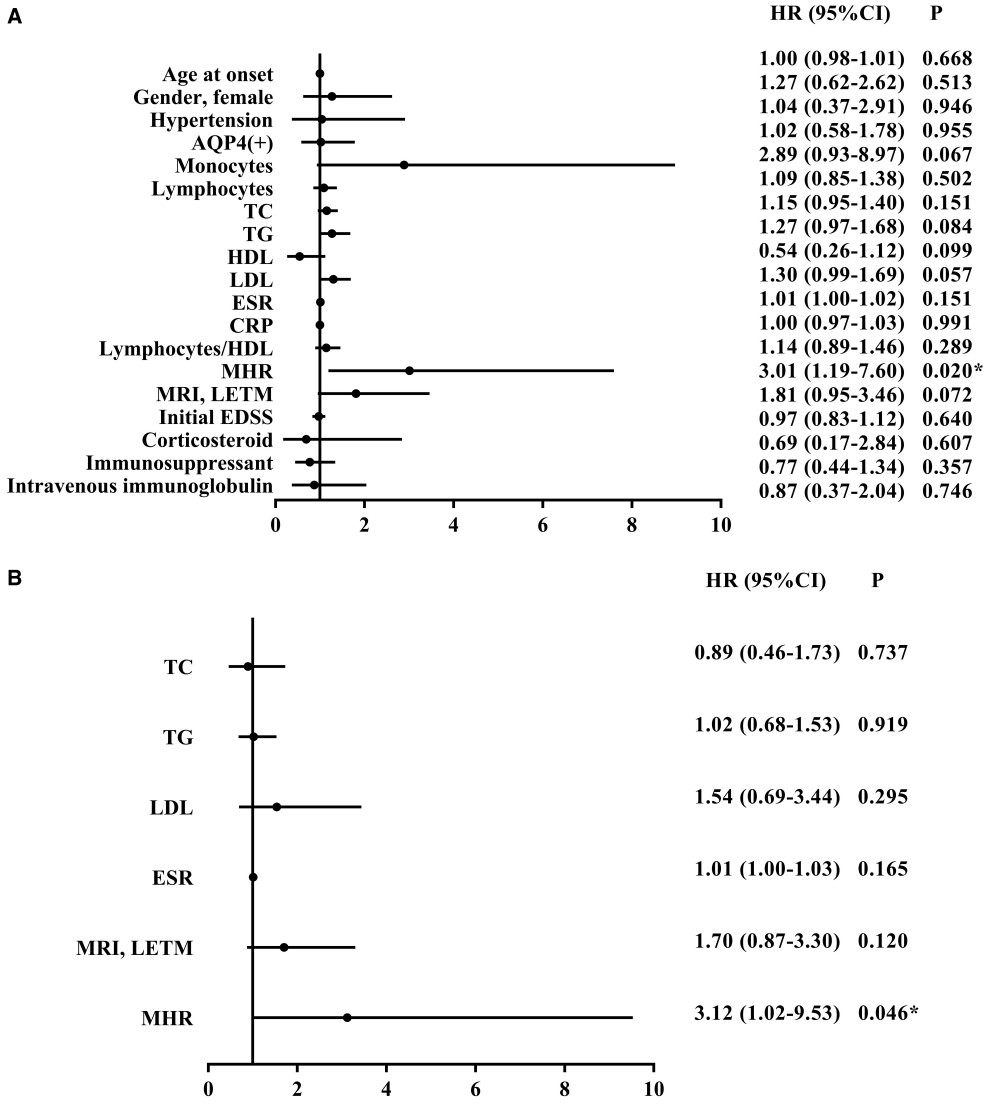

FIGURE 4 | Univariate **(A)** and multivariate **(B)** cox survival analysis of potential factors associated with relapse of NMOSD patients. NMOSD, neuromyelitis optica spectrum disorders; AQP4, aquaporin-4; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; MRI, magnetic resonance imaging; LETM, longitudinally extensive transverse myelitis; STM, short-segment transverse myelitis; EDSS, Expanded Disability Status Scale; MHR, monocyte to high-density lipoprotein ratio; HR, hazard ratio; CI, confidence interval. *$P < 0.05$.

anti-inflammatory cytokines (IL-10) decreases accordingly (40). Among these cytokines, IL-6 plays a key role in the pathogenesis of NMOSD, and treatment with IL-6 has shown clinical benefits in patients with NMOSD (41). Anti-AQP4 antibody is the main pathogenic antibody of NMOSD (42), but it alone is not enough to cause the disease. AQP4-specific T cells, especially Th17, can help peripheral blood B cells produce autoantibodies, induce tissue inflammation, and promote further injury to the central nervous system. IL-1β (43) induced by monocyte activation not only promotes Th17 differentiation but also causes blood–brain barrier leakage and enhances T cell migration (44); therefore, monocytes may play an important role in the pathological mechanism underlying NMOSD progression (45).

HDL is considered an anti-inflammatory factor that can prevent the production of pro-inflammatory cytokines and affect a series of immune cell reactions, including those involving macrophages and B and T lymphocytes (21). HDL is closely related to monocytes and can regulate the activation, adhesion, and migration of monocytes (46, 47). Apolipoprotein A-1, the main protein component of HDL, has a specific inhibitory effect on the production of inflammatory cytokines by monocytes through prevention of the activation of CD11b (48, 49). The

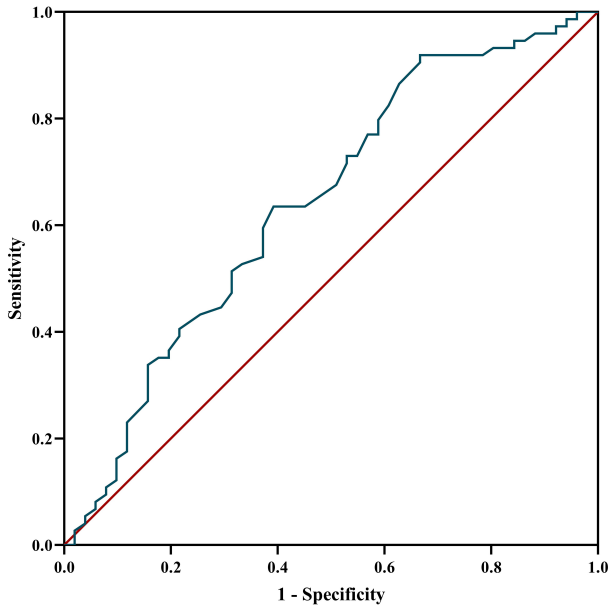

FIGURE 5 | ROC curve analysis of the prediction of relapse based on MHR. ROC curve, receiver operating characteristic curve; MHR, monocyte to high-density lipoprotein ratio.

production of anti-AQP4 antibodies in patients with acute NMOSD leads to the loss of astrocytes in specific areas of the central nervous system through complement-mediated cytotoxicity (50). It has been shown that astrocytes can produce apolipoprotein A-I (51) in rats; hence, the extensive loss of astrocytes leads to a significant decrease in the production of apolipoprotein A-I and a decrease in HDL levels. Previous studies have shown that the serum apolipoprotein A-1 levels in patients with NMOSD are significantly lower than those in healthy controls (22), and HDL levels are significantly lower during active disease than during remission. Further, dyslipidemia with low HDL is related to disease activity and disability in patients with AQP4 positive NMOSD (21). Similar results were observed in the present study. The lower the HDL levels, the higher the initial EDSS score of patients with NMOSD. Although TC, TG, and LDL had a moderate influence on disease prognosis in univariate analysis, no significant correlation was observed in multivariate analysis, and abnormal lipid metabolism in NMOSD has been confirmed by many studies. Some studies have found that compared with healthy controls, the levels of TC, TG, and LDL in patients with NMOSD are higher, and the level of TGs is positively correlated with poor recovery of first-time patients with NMOSD (20, 52). This, combined with our research results, suggests that early lipid-lowering treatment may play an important role in improving prognosis.

Compared with single monocytes and HDL levels, combining monocytes and HDL to form a new comprehensive inflammatory index that incorporates both the injury mechanism and protection mechanism, namely the MHR, may have greater clinical value as this biomarker can reflect the degree of inflammation and oxidative stress. Many studies have shown that MHR is closely related to Parkinson's disease (53), ischemic stroke (54), cardiovascular disease (23), metabolic syndrome (55), immune system disease (26), and rheumatic disease (27). Related studies have also reported that the neutrophil-to-lymphocyte ratio (NLR) was related to disease activity at the onset of MS (56). However, another study provided results that did not support the use of NLR as a marker of disease activity and disability in MS patients (57). The association between NLR and disease severity was also confirmed in NMOSD (58). However, the effect of MHR on the pathogenesis and prognosis of patients with NMOSD has not been previously reported. In our study, we found that MHR was positively correlated with the EDSS score of patients with NMOSD ($r = 0.306$, $P = 0.001$), and patients with high MHR had a higher relapse rate, which was an independent risk factor for disease severity and poor prognosis. Recently, it has been reported that the MHR of patients with multiple sclerosis with EDSS score ≥ 4 is significantly higher than that of patients with EDSS score < 4, which proves that MHR is related to the severity and disability of MS and can be used as an independent indicator to predict disability (31). This previous report, combined with our research, suggests the potential role of MHRs in autoimmune demyelinating diseases of the central nervous system, and can also be used for risk assessment in the diagnosis and treatment of NMOSD. In our ROC curve analysis, MHR > 0.565 was associated with a high risk of relapse; therefore, intervention in the early stage of the disease process to reduce the MHR may have therapeutic potential, improve disease prognosis, and reduce the risk of relapse in clinical practice.

In our study, univariate analysis showed that spinal cord MRI showing LETM had a moderate influence on the initial EDSS score and prognosis of NMOSD, but there was no significant correlation in multivariate analysis, and previous studies have found that the length of spinal cord lesions is related to the initial severity of the disease and residual disability (59). The ability of MRI parameters to predict NMOSD prognosis requires further exploration. Consistent with previous studies, we found that the CRP was closely related to the disease, and the CRP level was positively correlated with the initial EDSS score ($r = 0.237$, $P = 0.008$) (60). The serum CRP level of patients with NMOSD was significantly higher than that of healthy individuals, which may be related to the oxidative stress and inflammatory reactions that occur during disease pathogenesis (61). CRP level is also related to the destruction of the blood–brain barrier and is a useful index for monitoring NMOSD disease activity (60, 61).

Our study also has several limitations worth noting. First, the retrospective study design has inherent defects. Second, this was a single-center cohort study with a relatively short follow-up time; therefore, a larger multi-center study with a longer follow-up period is warranted to confirm our results. Third, our hospital did not evaluate MOG antibody levels prior to 2019, and the lack of data prior to this date may have affected the research results. Fourth, selection bias should be considered. Because of incomplete test data, loss to follow-up, and other reasons, we only studied a small number of patients (125/650). Finally, some uncontrollable confounding factors may have affected the research results, such as smoking, drinking, eating habits, and drug intake.

In conclusion, our results showed that MHR was correlated with the severity of early NMOSD and could further predict disease relapse. This new biomarker, which is simple, reliable, economical, and easy to obtain, can also be used for NMOSD risk assessment in clinical practice and may facilitate earlier treatment and improvement of prognosis. Further studies are required to elucidate the specific mechanisms underlying the effect of MHR on NMOSD pathogenesis and disease progression.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Zhengzhou University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JZ and YL contributed to conception and design of the research. YZho, CP, and YZha organized the database. JZ, YL, and HX performed the statistical analysis. JZ and KW conducted regular follow-up of all cases. JZ wrote the first draft of the manuscript. RD, ZG, and YJ undertook the task of revising the manuscript critically. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.763793/full#supplementary-material

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Impact of Andropause on Multiple Sclerosis

Maria C. Ysrraelit[*†] *and Jorge Correale*[†]

Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (FLENI), Buenos Aires, Argentina

Andropause results from the natural decrease in testosterone levels that occurs with age. In contrast to menopause, which is a universal, well-characterized process associated with absolute gonadal failure, andropause ensues after gradual decline of both hypothalamic-pituitary-gonadal axis activity, as well as of testicular function, a process which usually develops over a period of many years. Increasing evidence on greater risk of Multiple sclerosis (MS) associated with lower testosterone levels is being reported. Likewise, epidemiological studies have shown a later age of onset of MS in men, relative to women, which could perhaps respond to the decline in protective testosterone levels. In this review, we will discuss the role of androgens in the development and function of the innate and adaptive immune response, as well as in neuroprotective mechanisms relevant to MS. Testosterone effects observed in different animal models and in epidemiological studies in humans will be discussed, as well as their correlation with physical disability and cognitive function levels. Finally, published and ongoing clinical trials exploring the role of androgens, particularly at key stages of sexual maturation, will be reviewed.

Keywords: andropause, testosterone, aging, sex hormones, multiple sclerosis

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Hans-Peter Hartung,
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Jefferson Becker,
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Jorge Tolivia,
University of Oviedo, Spain

****Correspondence:***
Maria C. Ysrraelit
mcysrraelit@fleni.org.ar

[†] These authors have contributed equally to this work

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INTRODUCTION

Multiple sclerosis (MS) is a demyelinating immune-mediated disease of the central nervous system (CNS) universally more prevalent in women than in men, a phenomenon shared with several other autoimmune diseases. Indeed, different studies have reported greater MS prevalence in females, with the current female/male ratio estimated to be 3:1 (1). Important gender differences in inflammatory activity and progression of disease have also been observed. Male patients not only develop disease later, they experience less relapses. However, they also accumulate disability faster, reach milestones more rapidly, and show poorer recovery after initial exacerbations, compared to females (2). These findings have led to extensive studies on differences between the immune and nervous systems of men and women, in response not only to their specific gonadal hormones, but also to genetic factors, exposure to environmental factors and varying lifestyles (3). It has been hypothesized that the natural age-related decline in testosterone, the main male sex hormone, may play a role in these gender-related differences in MS prevalence and in clinical characteristics.

Several studies have highlighted the modulatory role of female sex hormones on MS disease activity during different stages of reproduction such as puberty, pregnancy and menopause. However, less is known about the impact of reproductive senescence in men. In contrast to menopause, which is a universal, well-characterized process associated with absolute gonadal failure, andropause is characterized by a gradual decline in hypothalamic-pituitary-gonadal axis activity, as well as decreased testicular function, occurring over a period of many years. Concentrations of bioavailable testosterone can decrease by as much as 50% between the ages

TABLE 1 | Main neuroprotective effects of androgens.

Effects of testosterone on the CNS	References
Improves survival of human neurons and astrocytes, inhibiting generation of reactive oxygen and nitrogen species.	(20, 21)
Upregulates of neuroglobin secretion by astrocytes and microglia after injury, glucose deprivation, and kainic acid toxicity.	(22–25)
Increases expression of neurotrophic factors such as brain derived neurotrophic factor (BDNF) which activates brain neurogenesis, dendritic spine maturation and modulates motor neuron morphology.	(26–28)
Stimulates of neural plasticity and neural differentiation	(29)
Promotes synaptic density and increase the growth of neurites	(30)
Increases connectivity of hypothalamic neurons	(31)
Reduces reactivity of astrocytes and reactive microglia following brain injury	(32)
Delays the aging process	(33)
Preserves excitatory synaptic transmission in the hippocampus during EAE	(34)

of 25 and 75 years (4). Given that in men there is no abrupt hormonal cutoff, or clear period of symptomatic change, some authors argue that the term "andropause" or "male menopause" is inappropriate, and that the phenomenon should be called late-onset hypogonadism (LOH) or age-related androgen decline (5). Dehydroepiandrosterone (DHEA), an adrenal precursor to more potent androgens and estrogens, as well as its metabolite DHEA sulfate (DHEA-S) also decline with age, at a rate of 3% per year, 3 times faster than testosterone, falling to one-third of serum concentrations by age 70 (6).

In this review we will discuss the role of androgens in the development and function of the innate and adaptive immune response, as well as neuroprotective mechanisms relevant to MS. Testosterone effects observed in different animal models and epidemiological studies in humans will be analyzed, as well as their correlation with physical disability and cognitive function levels. Finally, we will review published and ongoing clinical trials exploring the role of androgens, particularly at different stages of sexual maturation.

EFFECTS OF ANDROGENS ON IMMUNE FUNCTION

Andropause appears to contribute, at least in part, to immunosenescence, and consequently to progression of disability in MS. Immunosenescence is an age-associated decline in function of both the adaptive and innate immune systems. MS patients may experience premature onset of this phenomenon (7). In parallel, specific effects of androgens include a shift from Th1 to Th2 phenotype, based on increased production of IL-5 and IL-10, and decreased pro-inflammatory cytokines including IFN-γ, TNFα, IL-1, IL-6, and IL-17. Testosterone also reduces lymphocyte proliferation and differentiation and may suppress immunoglobulin production (8). Supra-physiological doses of testosterone also inhibit cytotoxic NK cell activity (9, 10). Therefore, androgens should, in principle, be considered anti-inflammatory hormones. In the CNS, dihydrotestosterone inhibits LPS-induced release of proinflammatory factors, including TNF-α, IL-1β, IL-6; iNOS, COX-2, NO, and PGE2 in BV2 cells and primary microglia cells, through suppression of TLR4-mediated NF-κB and MAPK p38 signaling pathways, thus protecting neurons from inflammatory damage induced by activated microglia (11). Similar changes have been observed in experimental *in vivo* models. In fact, castration of male animals has had detrimental effect on susceptibility to, and severity of EAE (12). MBP-specific T lymphocytes derived from the spleen of male animals during the effector phase of adoptive EAE, produced significantly higher levels of IL-10 (13), and treatment with testosterone was protective, an effect linked to androgen-mediated Th2 bias, as suggested by the IFNγ/IL-10 ratio (12, 13).

Similar to testosterone, DHEA inhibits transcription factor NF-κB, and suppresses secretion of IL-1β, TNF-α, and IFN-γ (14). In animal models, DHEA decreases T cell response and shows anti-inflammatory effect on microglia and astrocytes, ameliorating EAE severity and inflammation (15, 16).

In the thymus, the autoimmune regulator (Aire) gene prevents autoimmunity by promoting self-antigen expression in medullary thymic epithelial cells, such that developing T cells that recognize these self-antigens within the thymus, undergo clonal deletion. Androgens recruit androgen receptors to Aire promoter regions, enhancing Aire transcription. Thus, androgen levels in males may increase Aire expression to a degree that protects against autoimmunity. In line with this, in EAE mice, androgen administration as well as male gender, protected against autoimmunity in an Aire-dependent manner, indicating that control of intrathymic Aire-mediated tolerance mechanisms by androgens, contributes to explain gender-related differences observed in MS (17).

NEUROPROTECTIVE MECHANISMS

Testosterone crosses the blood-brain-barrier, directly influencing neuronal cells (18, 19). The main effects of testosterone on the CNS are summarized in **Table 1**. Similarly, in humans, DHEA has demonstrated neuroprotective effects, increasing neurite growth,

promoting neurogenesis and neuronal survival, influencing apoptosis, and catecholamine synthesis and secretion, as well as exerting antioxidant effects (6).

Recent studies have shown that testosterone may also play an important role in myelination processes. Indeed, after lysolecithin-induced demyelination, testosterone favors astrocyte recruitment and spontaneous oligodendrocyte-mediated remyelination (35). Similarly, castration of male animals results in decrease in myelination in the corpus callosum, both under normal conditions and after long term administration of cuprizone. These processes are reversed following exogenous testosterone administration (36).

In line with these findings, other studies have shown that testosterone, *via* specific involvement of androgen receptors, induces proliferation and differentiation of oligodendrocyte precursors (OPCs), as well as activation and proliferation of astrocytes and microglial cells (36).

CLINICAL FINDINGS

Andropause or LOH refers to the gradual and lifelong decline in serum testosterone concentration and testicular function that occurs with aging. Healthy older men will experience approximately 40% reduction in total Leydig cell mass over time. LOH will also likely be influenced by comorbidities associated with aging and the development of chronic illnesses, including obesity, diabetes, cardiovascular disease, and inflammatory disorders, all associated with accelerated aging-related testosterone decline. Presentation of MS symptoms in older patients will be further impacted by these events (37, 38).

Clinical characteristics of andropause include diminished sexual desire and erectile capacity, decreased intellectual activity, fatigue, depression, loss of muscle mass and body hair, anemia, decrease in bone mineral density resulting in osteoporosis, and increased visceral fat and obesity (39). These symptoms may overlap with the effects of aging, worsening the motor disability, fatigue, cognitive decline and psychiatric symptoms caused by MS (19).

Both MS symptoms as well as their severity also appear to differ between males and females. Men present later onset of disease (40, 41) and experience less frequent relapses with poorer recovery (42, 43). They show faster progression (Malik Neurology, 2014), worse outcomes (42) more cerebellar involvement, as well as greater cognitive impairment (42, 44). In line with these clinical findings, MRIs from men with MS show less inflammation (40), more gray matter atrophy (44) and more T1 lesions (45). Intriguingly, these clinical findings are not observed in pediatric MS cases prior to puberty (46), or in women with MS onset during menopause (around 50 years of age on average in Western societies), suggesting a more complex interplay between hormonal mechanisms related to reproductive senescence or aging, and the course of the disease (47). Young MS patients show slower progression of disability than adults or late onset MS patients, but experience relapses more frequently (48, 49). Risk of relapse seems to decrease continuously with time until patients reach the age of 35. In contrast, disability worsening remains stable from childhood to about 32 years of age, and then increases sharply after the age of 45 (50). Typical age of MS onset in men is around 40, coinciding with the physiological age-related decline in androgen levels, suggesting loss of testosterone could contribute to development of MS. Overlap with other

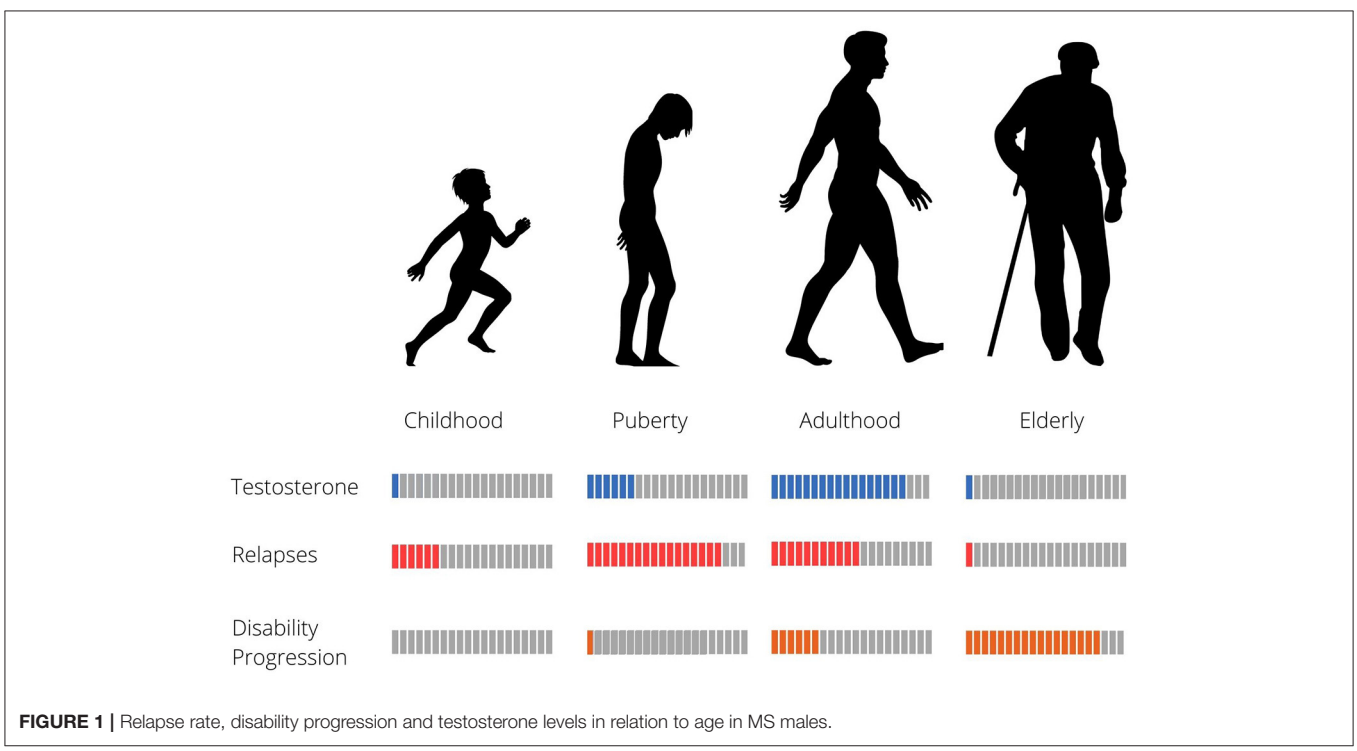

FIGURE 1 | Relapse rate, disability progression and testosterone levels in relation to age in MS males.

TABLE 2 | Main clinical trials using testosterone in MS.

Study Title	Interventions	Design	Main Outcome	Status	Comments
Testosterone Treatment for Multiple Sclerosis (NCT00405353)	Androgel: 10 grams of gel containing 100 mg of testosterone	Pilot cross-over 10 patients	Brain atrophy rate and Cognitive testing	Completed	One year of follow-up, shows improvement in cognitive performance and slowing brain atrophy.
Oral Testosterone for Fatigue in Male Multiple Sclerosis Patients (NCT01516554)	Testosterone undecanoate vs. placebo	Randomized controlled cross-over	Change in Modified Fatigue Impact Scale	Terminated due to poor recruitment	––
Testosterone Treatment for Erectile Dysfunction and Multiple Sclerosis (NCT04601233)	Testosterone 75 mg sub cutaneous using auto injector	Single open label Estimated enrollment: 20 patients Intervention period: 12 weeks	Self-reported erectile function measured by: ADAM Score SHIM Score MSHQ-SF.	Not yet recruiting	–-
TOTEM RRMS: TestOsterone TreatmEnt on Neuroprotection and Myelin Repair in Relapsing Remitting Multiple Sclerosis (NCT03910738)	Testosterone Undecanoate 1,000 mg vs. Placebo	Multicentric, randomized, parallel groups DBPC, Phase 2 Estimated enrollment: 40 patients Intervention period 66 weeks	Change on MRI binary criterion combining thalamic atrophy and modification in transverse diffusivity.	Recruiting	––

ADAM Score, Androgen Deficiency in the Aging Male; DBPC, Double blind placebo controlled; SHIM Score, Sexual Health in Men; MSHQ-SF, Sexual Health Questionnaire short form.

phenomena related to aging, such as immunosenescence could also influence patient symptom profiles. Effects of androgen levels and aging in men with MS are summarized in **Figure 1.**

Several studies have found lower testosterone levels in men with MS, compared to healthy age-matched subjects (51–53). In a cohort of 96 men with relapsing remitting MS, mean age of 40 years and disease duration <10 years, 39% of patients were hypogonadal with no compensatory rise in luteinizing hormone, suggesting central hypogonadism (54). Interestingly, the authors reported a correlation between low testosterone levels and disability (54). Other studies however, were not able replicate these findings (53).

An analysis of linked national Hospital Episode Statistics from England reported a strong positive association (5-fold elevation) between testicular hypofunction, as a proxy for low testosterone levels, and subsequent risk of MS in males (55).

Recently, increased risk of MS was reported in transgender individuals receiving estrogens and anti-androgens (56), suggesting influence of feminizing hormones, or low testosterone levels on risk of disease, providing further evidence of the importance of sex hormones in MS pathophysiology.

POTENTIAL USE OF TESTOSTERONE FOR MS TREATMENT

Testosterone replacement therapy is commonly indicated in aging and hypogonadal men. Hormonal supplementation induces virilization, improved libido and energy, increased muscle strength and fat-free mass, and strengthens bone density. Its use requires monitoring of prostate-specific antigen, as well as of hematocrit levels.

In a pilot clinical trial with cross-over design, 10 RRMS men aged <65 years, were treated with 100 mg of testosterone (6 months observation followed by 12 months of treatment). Treatment resulted in improvement of cognitive performance, and slowing of brain atrophy, with no significant effect on gadolinium-enhancing lesions (Gd) (57). Subsequent evaluations of this same study have shown testosterone treatment decreased CD4+T cells and IL-2 production, and increased NK cells as well as TGF-β1 secretion (8). Furthermore, voxel-based morphometry of the brain showed not only less global atrophy, but also significant increase in gray matter in a cluster in the right frontal cortex (58).

TOTEM RRMS (NCT 03910738) is a phase 2, multicenter, randomized placebo controlled, double-blind trial carried out in 40 testosterone-deficient men with relapsing-remitting MS, which aims to prevent MS progression. Patients will be randomized into two groups, to receive either testosterone undecanoate or placebo over a period of 66 weeks. All patients will be treated with natalizumab during the trial. The primary outcome is to measure the neuroprotective and remyelinating effects of testosterone using tensor diffusion imaging techniques and thalamic atrophy analyzes. Secondary outcomes include use of conventional MRI sequences as well as clinical parameters to assess cognition, fatigue, quality of life, impact on work activity and anxiety/depression. Recruitment is expected to end around September 2021 (59).

The main clinical trials using testosterone are summarized in **Table 2**. These trials underline the potential use of testosterone as an immunomodulatory, neuroprotective and remyelinating molecule. Importantly, testosterone did not cause significant side effects in any of these trials, suggesting this treatment could represent a safe adjunctive therapy for MS and other neurodegenerative diseases (19).

CONCLUSIONS AND FUTURE PERSPECTIVES

Age is one of the major determinants of the clinical course of MS. Aging mechanisms will likely affect multiple clinical aspects of the disease, as well as influence underlying pathological mechanisms, immunological changes, and treatment efficacy.

Notably, transition from RRMS to more progressive disease phases will overlap with the naturally occurring age-related decline of androgens in men, and with menopause in women. Clinical findings already suggest specific association between reproductive senescence and MS progression. We have described the influence of testosterone on MS and its potential effects on immunosenescence as well as on neuroprotection. However, further longitudinal studies on disease activity in diverse populations of men with MS are needed. Available information regarding DMTs in older men suffering from andropause and MS is limited, as clinical trials often restrict enrollment to those aged < 55 years. Understanding the complex interplay between reproductive senescence and the course of MS is of critical importance to elucidate mechanisms driving disease progression in older patients, and minimize progression of disability.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Immunosenescence and Autoimmunity: Exploiting the T-Cell Receptor Repertoire to Investigate the Impact of Aging on Multiple Sclerosis

Roberta Amoriello[1*], *Alice Mariottini*[2] *and Clara Ballerini*[1*]

[1] Dipartimento di Medicina Sperimentale e Clinica (DMSC), Laboratory of Neuroimmunology, University of Florence, Florence, Italy, [2] Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino (NEUROFARBA), University of Florence, Florence, Italy

T-cell receptor (TCR) repertoire diversity is a determining factor for the immune system capability in fighting infections and preventing autoimmunity. During life, the TCR repertoire diversity progressively declines as a physiological aging progress. The investigation of TCR repertoire dynamics over life represents a powerful tool unraveling the impact of immunosenescence in health and disease. Multiple Sclerosis (MS) is a demyelinating, inflammatory, T-cell mediated autoimmune disease of the Central Nervous System in which age is crucial: it is the most widespread neurological disease among young adults and, furthermore, patients age may impact on MS progression and treatments outcome. Crossing knowledge on the TCR repertoire dynamics over MS patients' life is fundamental to investigate disease mechanisms, and the advent of high-throughput sequencing (HTS) has significantly increased our knowledge on the topic. Here we report an overview of current literature about the impact of immunosenescence and age-related TCR dynamics variation in autoimmunity, including MS.

Keywords: multiple sclerosis, T cell receptor (TCR), disease modifying therapies (DMTs), aging, autoimmune diseases

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****Correspondence:***
Clara Ballerini
clara.ballerini@unifi.it
Roberta Amoriello
roberta.amoriello@unifi.it

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INTRODUCTION

Immunosenescence is a natural consequence of the biological process of aging. The immune system progressively declines throughout life: the involution of thymic activity begins with puberty and, as age advances, the regenerative potential of immune cells decreases, skewing the T- and B-cell compartment (1, 2). Such changes reduce the immune system reactivity, making the individual more prone to infections and developing cancer.

T lymphocytes are profoundly impacted by immune aging. Over time, the T-cell compartment gradually switches towards a homeostatic maintenance of the existing cells rather than generating new ones, as reflected by the reduction of naïve cells (3, 4). In these circumstances memory T cells become prevalent, showing changes in either immunophenotype (5, 6) and gene expression (7, 8). The composition of the memory T-cell compartment in advanced age is closely linked to the individual immunological history, e.g. the infections acquired during childhood and adolescence.

Viruses that infect the organism and then become lifelong latent have a major role in shaping the T-cell response and building-up the memory T-cell repertoire (9, 10). Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are two examples of pathogens that infect a massive part of the human population (global prevalence is up to 80% and 95% for CMV and EBV, respectively) (11, 12), leaving a signature within the immune system that is common to most of individuals and that, in some cases, alters the immune system composition as age progresses.

The application of high-throughput sequencing (HTS) demonstrated that age-dependent T-cell compartment depletion reflects the impairment of the T-cell receptor (TCR) repertoire diversity that appears reduced, leading to a narrowed antigen recognition potential breadth (4, 9, 13). Albeit this process naturally occurs in both health and disease, in the latter case it may exacerbate the pathological condition, weakening immune defense and capability of recovering (e.g. hampering to repair tissue damage and to heal from acute infections) and contributing to disease progression (14). This is particularly crucial in autoimmune disorders, in which the disruption of immune tolerance and immune aging are mutually related, as it has been observed in patients with Rheumatoid Arthritis (RA), who showed a disease-dependent premature and accelerated immunosenescence process (15).

Multiple Sclerosis (MS) is an inflammatory, systemic, heterogeneous disease in which autoreactive T cells migrate from the periphery to the central nervous system (CNS), leading to myelin disruption. MS is widely considered an autoimmune disease, however the antigen that triggers the abnormal immune response is still unknown. HTS is greatly contributing to our understanding of MS pathogenesis being a powerful tool to bridge molecular and clinical data, such as detecting longitudinal treatment-dependent signatures in the TCR repertoire of patients (16, 17). The relationship between aging and MS pathogenesis and progression is well known: immunosenescence and skewed T-cell compartment diversity in MS patients have been linked to a higher risk of developing a potentially fatal neurological disorder, the Progressive Multifocal Leukoencephalopathy (PML) (18); furthermore, patient's age differentially impacts treatment outcome (19, 20). A recent study investigated the TCR repertoire dynamics in MS patients of different ages and enlightened interesting results showing that, despite the physiological decline of TCR repertoire diversity with age, this does not significantly differ between MS and healthy people (21).

In this Review, we summarize the current knowledge about immunosenescence and age-related TCR repertoire dynamics in autoimmune diseases, with emphasis on MS, and how HTS shaped the scientific perspective on this investigation.

T-CELL RECEPTOR AGING IN HEALTH: OVERVIEW OF HIGH-THROUGHPUT SEQUENCING INVESTIGATION

The imprecise V-J genes rearrangement generates the TCRβ chain, for a theoretical amount of about 10^{15} TCRβ sequences and a real estimate ranging between 10^6-10^8 (2, 22). The high variability of the TCRβ molecule mainly lies in the complementarity-determining region 3 (CDR3), the first responsible of the bond, with variable affinity, between the receptor and the cognate antigen. For this reason, the amino acid CDR3 sequence (CDR3-a.a.) is the target of election of HTS investigation.

The process of thymic involution begins in the first years of life and progresses over puberty and adulthood (23, 24), determining a contraction of the T-cell compartment and a decrease of newly generated T cell: thus, naïve cells decline, memory T cells become prevalent, and TCR repertoire diversity dwindles (4, 25, 26). TCR repertoire richness starts to narrow noticeably from the age of about 40 (4); the naïve repertoire significantly declines in 70 years old (y.o.) adults, with 8-57 million different nucleotide sequences compared to the range of 60-120 million in young adults (20-35 y.o.) (2) (**Figure 1**: summary of aging's impact on TCRβ repertoire in health).

Before the advent of HTS, aging's impact on T-cell compartment was investigated in a large cohort of 156 healthy donors (HD) of different ages, by analyzing the frequency variation of T-cell receptor excision circles (TRECs), small molecules of circular DNA generated by thymic TCR genes rearrangement thus meaningful of T-cell maturation and used as markers of immunosenescence (27). The study reported that CD4+ cell compartment tends to be stable until the age of 70 years, then declines alongside with TCR diversity and TRECs frequency. Recent HTS studies (2, 4, 13) agree that the contraction of TCR repertoire richness, including a skewed peripheral Vβ family expression (8), is mainly observable in the naïve T-cell compartment. On the other side, effector memory CD8 + cells number increases with age, especially those cells that likely recognize latent viruses encountered over life by the majority of the population, such as CMV (2, 3, 28). Qi et al. showed that the age-dependent variation of effector memory T cells number in HD does not mirror the TCR repertoire richness dynamics, that does not significantly differ between young and elderlies, dissimilarly from what observed for naïve T cells (2). Accordingly, a previous study documented a linear decline of TCR repertoire diversity with age in peripheral blood naïve T cells of 39 HD (4). In fact, this decline was directly correlated with the percentage of naïve T cells, but not with the total count of circulating CD3+ cells, which did not show any age-dependent shrink. Britanova et al. observed that the oldest group of HD (average age of 82) was characterized by a broader TCR repertoire diversity compared to the group with average age of 62, suggesting that this molecular feature might be related with longevity. More recently, a longitudinal study (29) tracked the TCR repertoire dynamics over 20 years, in 6 HD of age ranging between 23-50 at the enrollment. The TCRβ gene was sequenced by HTS from peripheral blood CD4+ and CD8+ T-cell subsets for three times, about 10 years apart. According to other findings, TCR repertoire diversity dwindled more prominently in CD8+ cells, whereas CD4+ cells maintained a higher diversity constantly over time. Furthermore, authors found that the top of most frequent CDR3s-a.a. persisted over the whole period of observation (20 years), thus suggesting that a part of the TCR repertoire composition tends to remain stable over aging. Such findings may

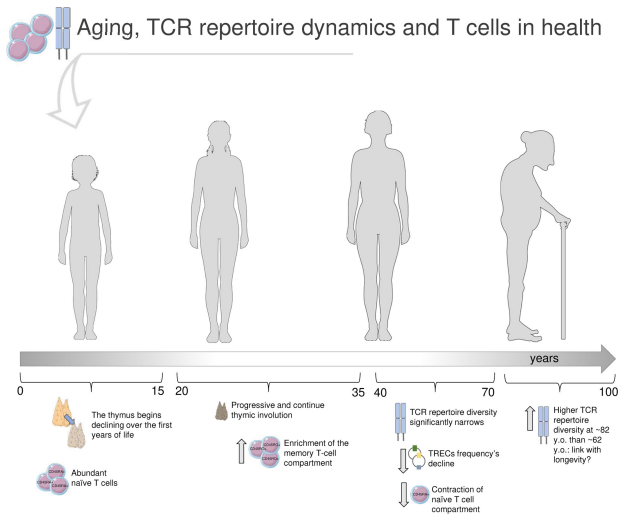

FIGURE 1 | Immunosenescence and T-cell receptor repertoire in health. In the first years of life, the thymus is at its maximum size and activity, and the T-cell compartment is characterized by abundance of naïve (CD45RA+) CD4+ and CD8+ cells. Earlier in life, the thymus starts to shrink, and continues to decline over puberty and adulthood, alongside with changes in the T-cell compartment, now enriched in memory (CD45RO+) cells (23–27). The TCR repertoire diversity significantly narrows after the age of 40 years old (y.o.) (1, 2, 4). The strongly reduced thymic output reflects in a decline of T-cell receptor excision circles (TRECs) frequency, small molecules of circular DNA representative of T-cell maturation (27). Furthermore, the naïve T-cell compartment results significantly dwindled (23–27). Through the latest stages of life, some TCRβ clones, especially across memory repertoires, are definitely predominant; interestingly, Britanova et al. documented a higher TCR repertoire diversity in older people (average age of 82 y.o.) compared to 62 y.o. individuals, that may be suggestive of longevity (4). Parts of the image created using pictures adapted from Servier Medical Art (https://smart.servier.com/).

indicate that the TCR repertoire is strongly impacted by the encounter of specific antigens, that contribute to skew the repertoire towards a higher clonality and predominance of some Vβ clones rather than others (where a "clone" is a V-J-CDRβ3 a.a. sequence); this may be particularly marked in CD8+ cells, first actors in pathogen epitopes recognition, whereas peripheral CD4+ cells could be mostly sustained by homeostatic proliferation. Egorov et al. (13) performed HTS on HD peripheral blood TCRβ repertoire and, according to Qi et al. (2), observed a reduction of TCR diversity. In addition, authors detected an age-dependent reduction of the average CDR3 length and of the number of N nucleotides that are randomly added during TCRβ generation, and such decrease was correlated with the age-dependent involution of naïve T-cell proportion. Interestingly, authors suggest that a contracted CDR3 length may impact peptide antigen interaction, shrinking the antigen recognition breadth of the elderly immune repertoire.

Less is known about the TCRγδ repertoire dynamics over life. TCRγδ cells account for about 4% of human circulating T cells and are mainly enriched in intestine and spleen tissue as intraepithelial lymphocytes (IELs) (30). Different Vγ families alternate from birth until advanced age, varying in proportion over different stages of life (31, 32). Similarly to TCRαβ, TCRγδ repertoire encounters a contraction of diversity and an increased clonality with age, especially in some Vγδ families such as Vγ9 +Vδ2+, and this is even prominent in naïve cells (33, 34). Since T γδ cells have important effector function in defense against pathogens and in bridging innate and adaptive immunity, their decline with age may contribute, along with T αβ cells, to a less active and less responsive immune system later in life.

SENESCENT T-CELL RECEPTOR REPERTOIRE IN AUTOIMMUNE DISORDERS: PROGRESSES AND PERSPECTIVES

T lymphocytes stage as main actors in a wide range of autoimmune diseases. Rheumatoid arthritis (RA), Celiac disease (CD), Diabetes Type 1 (T1D) and Multiple Sclerosis (MS) are main examples of autoimmune disorders in which T cells are crucial. In these diseases, the immune-mediated inflammation targets and damages different tissues, e.g. synovia in RA, small intestine in CD. Despite being a typical sign of autoimmunity, inflammation has been recently suggested to have a role in degenerative diseases as well, such as Alzheimer's disease (AD) and Parkinson's disease (PD) (35). HTS greatly contributed to characterizing the TCR repertoire in T-cell driven diseases and neurodegenerative ones (**Figure 2**).

Rheumatoid arthritis. Modifications in the T-cell compartment and TCR dynamics in RA are known since pre-HTS age (36). Synovial lesions infiltrates include mainly CD4+ T cells, that are characterized by an oligoclonal TCR repertoire and a narrowed TRBV families distribution (37–39). These findings have been recently implemented by HTS: Jiang et al. showed that RA patients share common features in their TCR repertoire, e.g. clonal expansion in the effector memory T-cell compartment and in T helper 17 (Th17) cells, and TCR diversity has been correlated with RA disease activity (40). Furthermore, authors detected abnormalities in the V-J gene usage that are shared between peripheral blood and synovial TCR repertoires, suggesting that autoreactive T cells might be activated and selected in the periphery and then infiltrate joints and *in situ* expand and contribute to inflammation. Immune aging has been discussed as a key factor in RA, despite contradictory findings being reported (41). T-cell compartment involution seems to be accelerated in RA patients, with increase of senescent CD28- T cells, characterized by declined proliferation and telomerase activity (42–44). In RA patients it was also documented a reduction of TRECs frequency; however, this was observed to be age-independent (45, 46), stressing the need for further investigation.

Celiac disease. CD is an autoimmune disease affecting the small intestine in which immune cells abnormally react against gluten proteins, causing inflammation and villous atrophy. Despite being a multifactorial disease, genetics plays a major role in CD susceptibility, in particular the human leukocyte antigen (HLA) DQ2 and DQ8 alleles, that are carried by up to 95% of patients (47, 48). CD4+ Th cells that react against gluten epitopes presented by

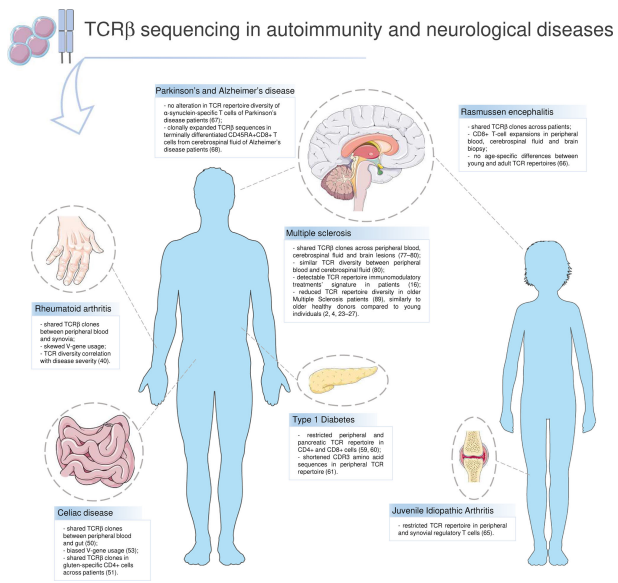

FIGURE 2 | T-cell receptor repertoire in autoimmunity and neurological diseases. Highlights on current knowledge about the TCR repertoire and the T-cell compartment in autoimmune diseases (Rheumatoid arthritis, Celiac disease, Type 1 Diabetes, Multiple Sclerosis) and in other disorders in which T lymphocytes have a role (Rasmussen encephalitis, Juvenile Idiopathic Arthritis, Parkinson's and Alzheimer's disease). Parts of the image created using pictures from Servier Medical Art (https://smart.servier.com/).

HLA-DQ2.2, HLA-DQ2.5 and HLA-DQ8 are considered as main actors in CD pathogenesis and have been found in both peripheral blood and small intestine (49). HTS gave an extensive contribution gaining insights into T cells pathogenic role and TCR repertoire dynamics in CD patients. Comprehensive analysis on TCRVβ sequencing from CD patients documented the presence of sharing Vβ clones between peripheral blood and gut TCR repertoires (50) and the persistence of gluten-specific clones for decades in the TCR repertoire of patients upon gluten-free diet (GFD) (51). HTS on single-cell gut IELs TCRγδ showed that CD patients, either under GFD or not, have a biased TRDV pattern compared to healthy controls, a private CDR3δ repertoire, whereas CDR3γ clones are shared between CD and controls (52). A recent investigation leveraging a large TCRαβ dataset of data from 63 CD patients (53) identified a number of 325 public TCRαβ clones in gluten-specific CD4+ T cells across patient; furthermore, they observed a biased V-gene usage and conserved CDR3α:CDR3β motifs across CD repertoires. Taken together, these findings indicate that TCR repertoire shares common features across CD patients that may be linked to disease pathogenesis and progression; however, the association between TCR repertoire dynamics, T-cell senescence and aging in CD is still not deeply investigated. First, CD has long been considered almost exclusively a pediatric illness, whereas it can show an adult-onset; second, due to the wide variety of symptoms that can be subtle in some cases, CD remains an underdiagnosed disease (54). Therefore, future insights on TCR dynamics on CD patient cohorts of different ages are needed.

Type 1 Diabetes. T1D is an autoimmune disease of multifactorial and not yet totally understood etiology, in which autoreactive T cells attack pancreatic β-cells hampering insulin production and causing hyperglycemia, with a wide set of signs and symptoms of variable severity. Increased susceptibility to T1D is given by environmental (body mass index, nutrition habits, total weight, etc.) and genetic factors (55), the latter sustained by the comorbidity in T1D patients with other autoimmune diseases (e.g. CD) and the association with certain HLA class II haplotypes, such as HLA-DRB1, HLA-DQA1, and HLA-DQB1 (56). T1D onset is usually before the age of 40 years, with a peak between 15 and 20 years old, but it can occasionally be diagnosed in older patients (57). Notably, older (>40 y.o.) patients may show aggravated signs and symptoms compared to younger cohorts, and require special management, in particular for consequences of hyper- or hypoglycemia, cognitive impairment, and chronic pain (58). Tong et al. have been among the first investigating TCR repertoire in T1D patients by HTS (59), leveraging a TCRβ dataset from peripheral blood of nine T1D, four Type 2 Diabetes (T2D) and six non-diabetic controls. Authors described a skewed V-gene usage in T1D and a higher proportion of "highly-expanded clones" in T1D compared to other groups, considering "highly expanded" those T-cell clones with a frequency≥1% of total reads in a repertoire. In the same year, Seay et al. sequenced the TCR repertoire in different T-cell populations from various compartments, e.g. peripheral blood and pancreatic draining lymph nodes (pLN) (60). Differentially from what observed from Tong et al., Seay et al. did not find any skew of the V-gene usage in T1D compared to other groups; they described instead a restricted repertoire in CD4+ cells and a certain proportion (24%) of shared CD8+ clones among tissues. One year later, Gomez-Tourino et al. published a study in which the TCR repertoire was sequenced from peripheral T-cell subpopulations of 14 T1D patients and 14 HD (61); authors detected abnormalities in CDR3s-a.a. length of T1D, in particular highly frequent shortened CDR3s-a.a. shared across T1D repertoires. This abnormal sequence length, authors suggested, may depend on early events in thymic selection in T1D, and shortened CDR3s-a.a. may facilitate the erroneous recognition of self-antigens. In the abovementioned studies, however, the enrolled cohorts have a quite homogeneous average age, and TCR repertoire dynamics is not described from an age-related perspective.

NEURODEGENERATIVE AND T-CELL MEDIATED DISEASES AFFECTING YOUNG OR ELDERLY

Juvenile Idiopathic Arthritis and Rasmussen Encephalitis

It is worth mentioning a couple of comprehensive and recent TCR repertoire HTS studies that were performed in diseases usually affecting the pediatric population and in which T cells have an established role, despite these diseases are not frankly

classifiable among autoimmune ones. This is the case of Juvenile Idiopathic Arthritis (JIA) and Rasmussen encephalitis (RE). The first is a rheumatologic disease with usual onset before the age of 16 years and of unknown etiology, in which pathogenesis T cells surely contribute, as demonstrated by the presence of activated T cells in synovial fluid and the effectiveness of treatments targeting T cells (62). RE is a progressive, chronic and inflammatory brain disease that mainly affects 6-7 y.o. children. RE pathogenesis is controversial and still under debate; however, it has been documented the presence of T cells, mainly CD8+, infiltrating the brain of patients (63, 64).

In JIA patients of age ranging between 4.9 and 15.1 years, the TCR repertoire of regulatory T cells (Treg) was restricted and expanded in both peripheral blood and synovial fluid (65). In RE patients, Schneider-Hohendorf et al. described CD8+ T-cell expansion in peripheral blood, cerebrospinal fluid (CSF) and brain biopsy, and Vβ clones and V-genes shared across patients (66). Authors analyzed TCR repertoire dynamics dividing patients between early (age range of 3-16 years) or adult (age range of 19-59 years) onset, but did not detect any age-specific difference between the two groups.

Parkinson's and Alzheimer's Disease

PD and AD are two neurodegenerative disorders mainly affecting the older part of the population, with an average onset of 60 y.o. for PD and over 65 y.o. for AD, in which the potential pathogenic role of T cells has been recently discussed (35). The TCR repertoire was investigated in T cells reactive to self-antigen α-synuclein (α-syn), associated with the disease, from six PD patients, and compared with the repertoire of T cells reactive to pertussis, encountered over life by most of the population (67). Interestingly, the TCR repertoire diversity of PD was similar to pertussis-reactive T cells. In AD patients, the TCR repertoire was recently investigated by Gate et al. who published their data in 2020. By single-cell technology, they sequenced the TCR repertoire from effector memory terminally differentiated (Temra) CD45RA+CD8+ T cells of AD CSF and found clonally expanded clones that are probably responsive to EBV antigens (68).

It is thus clear that some of the mentioned diseases differ for incidence, severity and progression based on age ranges within the population, and that TCR repertoire investigation by HTS widened our understanding of underlying mechanisms; however, the link with age is not established or not discussed in most of TCR studies, and further insights are required.

T-CELL RECEPTOR REPERTOIRE AND AGING IN MULTIPLE SCLEROSIS

MS is currently the most widespread potentially disabling neurological disease among young adults, with an average age of onset of 35 y.o (69). MS can manifest through different patterns, of which the Relapsing-Remitting (RRMS) is the most common one (85% of all MS diagnosis) and characterized by a relatively more benign course compared to Primary-Progressive MS (PPMS) onset. Usually, younger patients show a RRMS course; furthermore, women are affected more often than men, with a ratio of approximately 3:1 (70). Despite the undetermined etiology, it is known that an interplay of environmental and genetic factors contributes to MS susceptibility, the first including infections acquired during childhood and adolescence (e.g. EBV and CMV), smoking, vitamin D deficiency, whereas HLA genes are well-known among genetic factors, such as DRB1*15:01 and DRB5*01:01 alleles in European population (71).

T lymphocytes have an established role in MS pathogenesis (72). It is believed that T cells activated in the periphery migrate through the blood-brain barrier (BBB) into the CNS, leading to demyelination. Since the antigen that triggers the autoreactive T-cell response is still unknown, the investigation of TCR repertoire has been a great point of interest years before the advent of HTS. The first TCR studies were conducted exploiting flow cytometry analysis on Vβ families or spectratyping technology, that allowed to investigate TRBV families clonal prevalence in terms of CDR3 length distribution, but not at sequence level. Pre-HTS studies detected a skewed TRBV families distribution in MS peripheral blood (73), clonally expanded CD8+ T-cell clones in MS brain lesions (74), CSF (75, 76) and blood (76). Later HTS studies allowed to track the presence of shared CDR3s-a.a., in MS across peripheral blood and CNS compartments (CSF and brain) (77–79). In particular, researchers found expanded Vβ clones, especially in the CD8+ cell compartment, being shared across blood, CSF and brain lesions of patients (78, 79). Accordingly, a recent comprehensive analysis pooling MS TCR sequencing data from published and unpublished studies (80) showed that blood, CSF and brain lesions share sequences, despite CSF repertoire is overall more private compared to the periphery; furthermore, CSF and blood are quite similar in terms of TCR repertoire diversity, that does not significantly differ between the two compartments.

Several studies suggested that MS pathogenesis may be linked to premature immunosenescence, and that aging could in turn impact disease progression, severity, and treatment outcome. This topic has been extensively reviewed by Dema et al. (81), who recapitulated recent findings about aging and MS: patients showed signs of premature immunosenescence such as shortened telomeres (82), thymic dysfunction (83), decreased TRECs frequency (84), and accumulation of CD4+CD28- T cells (85). In this frame, Dema et al. also discussed therapeutic strategies based on rejuvenating senolytics that showed promising results in mice (86, 87).

Recently, Hayashi et al. (21) characterized the TCR repertoire by HTS in peripheral blood of 39 MS and 19 HD by using the newly developed Grouping of Lymphocyte Interactions by Paratope Hotspots (GLIPH), a clustering method that allows to group data based on chosen parameters, e.g. TCRVβ chain distribution or HLA haplotype. To avoid any potential treatment-dependent bias on the TCR repertoire, authors excluded from the study patients undergoing treatments that may perturbate the TCR repertoire, such as fingolimod and natalizumab. These two treatments act specifically on T cells:

the target of fingolimod is the sphingosine-1-phosphate receptor (S1PR), that is fundamental for T cells egress from lymph nodes and interacts with the C-C chemokine receptor type 7 (CCR7), involved in T cells homing to secondary lymphoid organs. Fingolimod induces the internalization of the S1PR, that binds CCR7+ T cells and causes their retention within lymph nodes. Natalizumab is a humanized monoclonal antibody targeting the integrin α4β1, or very late antigen-4 (VLA4), and blocking transmigration of T lymphocytes across the BBB. Both treatments are known to have immunomodulatory effects on T cells phenotype and TCR repertoire (ref. par. 5). In Hayashi et al., most (66.7%) of the enrolled patients were free from any therapy; the rest were under interferon-beta (20.5% of patients), prednisolone (10.5%) and azathioprine (2.6%). Authors analyzed data with regard to age, comparing MS with HD (age ranges of 40-63 and 43-59, respectively), and showed that older participants were characterized by lower TCRαβ and γδ diversity, with no significant differences between HD and MS. Interestingly, MS repertoires showed an overall age-independent broader TCR diversity compared to HD; such findings are in agreement with a previous study reporting a higher TCR diversity in MS in respect to HD and to a group of patients affected with a non-autoimmune neurological inflammatory disease, the Human T-cell leukemia virus type 1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) (88).

DISEASE-MODIFYING THERAPIES AND T-CELL RECEPTOR REPERTOIRE IN MULTIPLE SCLEROSIS

Disease-modifying therapies (DMTs) for MS encompass immunomodulatory or immunosuppressive treatments reducing the auto-reactivity of the immune system and promoting an anti-inflammatory environment (89, 90). Main data collected so far on DMTs, TCR repertoire and MS are summarized in **Table 1**.

Data on the impact of DMTs on TCR repertoire were first provided by studies on patients treated with autologous hematopoietic stem cell transplantation (AHSCT). AHSCT is a one-shot treatment (e.g. the administration of any DMTs is not required following the procedure, unless a disease reactivation is observed) consisting of four main steps. Briefly, hematopoietic stem cells (HSCs) are mobilized from the bone marrow by the administration of granulocyte-colony stimulating factor associated with cyclophosphamide (mobilization). The hematopoietic stem cells are then collected with leukapheresis and reinfused following the administration of high-dose chemotherapy (conditioning). Different drugs can be administered as a conditioning protocol, and conditioning regimens are classified in three grades of intensity (low, intermediate or high), according to the increasing grade of immunoablation induced (100). The immunoablation and the subsequent reconstitution promoted by the reinfusion of the HSCs induce a renewal of the immune system with a restoration of the immune tolerance. This latter phenomenon explains the long-term suppression of new inflammatory disease activity (relapses, and new T2 or gadolinium enhancing lesions) observed following transplant, in the absence of any maintenance therapy (101, 102). AHSCT has recently been endorsed as "standard of care" for the treatment of highly active RRMS failing DMTs by the European Committee for Blood and Marrow Transplantation (EBMT) guidelines (100), and its superior effectiveness compared to DMTs in RRMS patients was recently demonstrated by a randomized clinical trial (103). Younger age at treatment was independently associated with a reduced risk of disability progression following AHSCT in a retrospective multicenter cohort study (17), but this association is probably due to the predominance of different main drivers of disability progression according to age at treatment (i.e. disability accrual mostly inflammatory-driven in younger individuals than neurodegeneration driven in older individuals), rather than to a potential age-related variations in immune reconstitution following AHSCT.

The extensive study of variation of the TCR repertoire in patients treated with AHSCT provided valuable insights into the mechanism of action of the procedure, suggesting that the procedure was able to induce a re-booting of the immune system. Analysis of TRECs suggested that thymic reactivation could take place in adult individuals undergoing AHSCT, with the generation of new T cells following positive selection and maturation in reactivated thymus. Moreover, the reconstitution of an overall broader clonal diversity and an extensive renewal of clonal specificities compared with the pre-transplant assessment was first reported adopting CDR3 spectratyping (92). In a more recent study, HTS was applied to sequencing the TCRβ chains of 1 million sorted CD4+ and CD8+ T cells from each patient before transplant and 1 year after transplant (91). Impact of the procedure was different on CD4+ and CD8+ T cells: in CD4+ T cells, dominant TCR clones present before treatment were undetectable following the reconstitution, and patients largely developed a new repertoire. In contrast, dominant CD8+ clones were not effectively removed, and the reconstituted CD8+ T cell repertoire derived from clonal expansion of cells already detectable before treatment. Notably, patients who failed to respond to treatment showed less diversity in their TCR repertoire early during the reconstitution process, suggesting that this step is crucial for the successful outcome of the procedure. More recently, HTS was applied in paired blood and CSF samples from patients treated for active RRMS, comparing the reconstitution of T cell composition in both compartments before AHSCT and up to 4 years following transplantation (104). More than 90% of the pre-existing CSF repertoire was removed following AHSCT and replaced with Vβ clones predominantly generated from engrafted HSCs. Of the pre-existing clones in CSF, approximately 60% were also detected in blood before therapy, and concordant treatment effects were observed for Vβ clones in both compartments following AHSCT. Overall, these results indicate that replacement of the pre-existing TCR repertoire in active RRMS is a mechanism for AHSCT efficacy, suggesting that TCR analysis

TABLE 1 | Disease-modifying treatments, TCR repertoire and immunosenescence in Multiple Sclerosis.

Treatment	Route of administration	Mechanism of action	Effect on TCR repertoire	Link with immunosenescence
AHSCT	One-shot treatment consisting in (1) mobilisation of HSC from bone marrow (2); conditioning: leukapheresis and reinfusion of HSC after immunoablation by chemotherapy.	Immune system renewal and immune tolerance restoration.	- Broader TCR diversity (16, 91); - Effective reconstitution of CD4+ TCR repertoire (91); - Persistence of clonally predominant CD8+ T-cell clones (91); - Narrowed TCR diversity (91) and lower clonal persistence at 24 months from transplantation (16) correlated with treatment outcome and disease activity; - Public TCRβ clones shared across AHSCT patients after 24 months from treatment (16).	Increase of TRECs frequency and thymus reactivation after AHSCT (91, 92).
Alemtuzumab	Infusion in two cycles, one year apart.	Binding of CD52 with depletion of T and B cells and consequent repopulation.	Restricted TCR repertoire, especially in CD8 (93, 94).	- Reduction of TRECs frequency at 6 months of treatment, then return to basal levels; no TRECs-age correlation detected (95).
Glatiramer acetate	Subcutaneous injection daily or thrice a week.	Immunomodulant mimetic of myelin basic protein, attracting autoreactive T cells	No significant differences in TCR repertoire restriction compared to HD or pre-treatment (95).	Not documented.
Fingolimod	Oral, daily.	Causes S1PR internalization and retains T cells within lymph nodes.	Increasing TCR restriction over 12 months of treatment (spectratyping analysis (96);	Reduction of TRECs and KRECs frequency (95, 96); KRECs frequency, but not TRECs, was positively correlated with age (97).
Natalizumab	Infusion every four weeks.	Binding of VLA-4 integrin, blocking T-cell migration through the BBB.	- Oligoclonal TCR repertoire in CSF of treated patients (spectratyping analysis (98); possible connection with impaired CNS immuno surveillance and higher risk of PML; - Persistence of clones, especially memory CD8+, in peripheral blood of RRMS afer 24 months of treatment (16); - Broader TCR sequence similarity architecture in peripheral blood compared to one-shot treatment (AHSCT) after 24 months of natalizumab (16).	- Increase of TRECs frequency at 6 and 12 months of treatment, positively correlated with age (95); - Restricted TCR repertoire and decreased expression of CD49d, more pronounced in older patients (99).

might be adequately performed in peripheral blood as a surrogate for CSF.

The impact of AHSCT on TCR repertoire was furtherly investigated and compared with natalizumab in a recent HTS study (16): 15 RRMS patients' TCR repertoire dynamics was monitored longitudinally, before and after 24 months from AHSCT or under natalizumab, in peripheral naïve and memory T-cell subpopulations. The investigation detected treatment-specific signatures in RRMS TCR repertoire; in particular, authors found that AHSCT and natalizumab differentially impacted on TCR clonal expansion state, clonal persistence over time, and TCR repertoire architecture, and that such effects are traceable by comprehensive molecular approaches.

Accordingly with findings in MS (91, 104), successful AHSCT outcomes were correlated to an increased TCR repertoire diversity also in systemic sclerosis (SSc) (105, 106), a skin autoimmune disease, whereas SSc patients who experienced post-AHSCT relapse showed a reduced TCR repertoire diversity (107).

A few studies investigated the variation of TCR repertoire in MS patients treated with other DMTs such alemtuzumab, fingolimod, natalizumab and glatiramer acetate. Two studies reported data on thymic output and TCR repertoire in patients treated with alemtuzumab (93, 94, 108, 109). Alemtuzumab is a humanized monoclonal antibody targeting CD52, a surface marker primarily expressed on T and B lymphocytes, and inducing lymphocytic depletion followed by subsequent repopulation (109). Alemtuzumab is administered in two cycles, one year apart, and a re-treatment during year 3 might be administered in cases with persistent disease activity. In both the studies on alemtuzumab, TREC numbers were reduced following each course of treatment. The TCR repertoire was explored by CDR3 spectratyping and a more pronouncedly constricted TCR repertoire compared to baseline was detected following each cycle, and this was more pronounced in CD8+ cells (93). These data indicate that repopulation of T cells following the depletion induced by alemtuzumab is promoted by homeostatic proliferation of cells that have escaped depletion, rather than by newly generated cells from the thymus, suggesting that thymopoiesis is not significantly induced by alemtuzumab treatment.

Similarly, a narrowed TCR diversity in peripheral CD8+ cells of alemtuzumab-treated RRMS patients has been recently confirmed by a HTS study (94); these patients were characterized for the presence of highly active CD8+ cells in peripheral blood and infiltrating derma and developed vitiligo 14, 18 and 52 months after starting the treatment, therefore suggesting that the kinetics of B cells reconstitution and narrowing of the TCR repertoire might be involved in the development of secondary autoimmunity observed following alemtuzumab treatment (93, 94).

Fingolimod is a DMT that successfully reduces relapse rate and disease activity in relapsing MS (110). A longitudinal CDR3 spectratyping investigation detected the presence of TCR restrictions in peripheral blood of MS patients even before starting the treatment, which were then increased after 12 months of fingolimod (96). Furthermore, the study reported a significant reduction, over treatment, of TRECs and K-deleting recombination excision circles (KRECs) frequency in peripheral blood of patients.

A study adopting CDR3 spectratyping on patients treated with natalizumab (111) showed that during treatment patients exhibited a lower proportion of Vβ elements with TCR repertoire expansions in blood compared to non-natalizumab treated MS patients, but this phenomenon appeared to reverse in cases who developed PML (98). In the CSF, the TCR repertoire was more skewed or oligoclonal compared to corresponding blood samples, and these alterations in CSF were more prominent in patients treated with natalizumab compared to non-natalizumab-treated MS patients. The marked restriction of the TCR repertoire in the CSF of natalizumab-treated patients was suggested to critically weaken CNS immune surveillance exerted by patrolling memory T cells, potentially promoting the onset of PML in a proportion of John Cunningam virus (JCV) positive patients.

On the other hand, no significant differences by CDR3 spectratyping were detected in TCR repertoire of patients treated with glatiramer acetate compared to the pre-treatment, and the small variations reported in MS patients following start of treatment with glatiramer acetate were similar to those observed in healthy controls in the same study (112).

The potential additive effect of DMTs on premature immunosenescence and aging in patients with MS was evaluated in a few studies, exploring correlations with adverse events.

Paghera et al. quantified TRECs and KRECs in 122 MS patients aged from 17 to 60 years who had started therapy with interferon-beta, fingolimod, alemtuzumab, or natalizumab, measured in samples obtained before the therapy and at months 6 and 12 of treatment (95). TRECs and KRECs were used as surrogate markers of a senescent phenotype, as they are considered as indicators of thymic and bone marrow output (97). In therapy-naïve patients, the number of newly produced T and B cells was inversely correlated with age. The DMTs analyzed induced opposite changes in the production of new T and B cells, aligned with the known mechanism of action. The abovementioned correlation found at baseline was still detectable at month 12 of therapy with interferon-beta or natalizumab. On the other hand, both the correlations were lost in patients treated with alemtuzumab due to the reduction in TRECs and increase in KRECs, while in fingolimod-treated patients, only the correlation between TRECs and age disappeared. Overall, these data suggest that some DMTs might accelerate the immunosenescence of T cells, with potential increase of side effects mostly in elderly patients, aligned with the observation of higher risk of opportunistic infections during treatment in this latter population compared to young individuals (113).

Furthermore, the potential additional effect of DMTs on immunological changes induced by ageing has been suggested to play a role in the development of PML in MS patients receiving DMTs (18). The restriction of the TCR repertoire induced by natalizumab, further narrowing the TCR restriction induced by age, and associated with the impairment of T cells patrolling the CNS induced by the reduced transendothelial migration due to a decreased expression of CD49d (99), might in part explain the higher risk of PML and the worse outcomes reported in older patients compared to younger ones (114). Increased risk of PML in patients receiving fingolimod was associated with immune system changes induced by the treatment similar to those occurring during aging, as a predominance of Temra over naïve T cells (115, 116).

Collectively these data suggest that the risk of opportunistic infectious adverse events is increased in individuals with evidence of immunosenescence and that DMTs might exert an additional effect on the immune system of such individuals, considerably increasing this risk. Moreover, as DMTs might induce long-term effects on the immune system, persisting also following drug discontinuation, the actual immunological age of the individual might be persistently affected by previous treatments, thus making treatment switches challenging.

DISCUSSION

Autoimmune diseases are widely spread worldwide and affect people of all ages. Tracking TCR repertoire dynamics in humans is crucial to shed light on immunosenescence and its role in disease progression and treatments outcome; in this respect, HTS is greatly contributing.

To date, the majority of HTS studies on TCR repertoire in immunosenescence has been performed in healthy individuals. Recent findings highlighted that the healthy TCR repertoire is strongly impacted by antigens encountered over lifetime and consequently dwindles (3, 4, 10, 12), and such variations can be better appreciated in memory T cells after the age of 40 (4); furthermore, the shrinkage of TCR repertoire diversity correlates with the natural involution of the naïve T-cell compartment (4, 7, 13). TCR repertoire analysis in elderly also showed great potential unrevealing molecular markers of longevity, as Britanova et al. (4) found that healthy people older than 80 years are characterized by a broader TCR repertoire diversity compared to individuals of 65 y.o.

On the other hand, the current knowledge about immunosenescence and TCR repertoire dynamics in autoimmune and T-cell mediated diseases is still quite fragmented. In RA (15, 36–39), CD (49–53) and T1D (59–61) patients, different compartments (blood, synovia, gut, pancreatic draining lymph nodes) share TCRβ clones and show reduced TCR repertoire diversity. Furthermore, immunosenescence seems to be accelerated in RA patients (15, 42–44), and T1D severity is aggravated in older patients (58); however, most of these studies are not centered on an age-related perspective, stressing for further insights. It is worth mentioning that in Rasmussen encephalitis, a mainly pediatric disease of uncertain etiology but in which T cells have an established role, no correlation was found between TCR repertoire dynamics and patients' age (66).

In MS, the relationship between the TCR repertoire and aging is complex and distinguishes patients undergoing treatments that do not perturbate the TCR repertoire (e.g. interferon-beta) and patients under DMTs. In the first case, the TCR repertoire diversity declines with age similarly to HD, suggesting that the age-dependent involution of the T-cell compartment may not be among hallmarks of MS (21). On the other hand, variations of the TCR repertoire are observed during physiological aging and might be induced by the administration of DMTs in people affected by MS. The generation of a different and wider TCR repertoire compared to that one detected prior to treatment has been widely demonstrated in MS patients treated with AHSCT, and this is thought to mediate the therapeutic effect of the procedure through the reconstitution of a newly tolerant immune system, promoted also by the reactivation of thymopoiesis (91, 92). Alemtuzumab induces further restriction of the TCR through a homeostatic proliferation-based immune repopulation (93, 94, 109). Divergent effects on the TCR and TRECs are induced by other DMTs (96, 98, 112). All these observations were reported in the general MS population; to our knowledge, no data are available so far on the impact of DMTs on TCR in aging, and the differential effectiveness of DMTs across classes of age is mainly attributable to the different mutual relationship between inflammation and neurodegeneration underlying the accrual of disability.

The effect of DMTs on the immune system of MS patients, promoting in some cases the development of changes similar to those induced by physiological immunosenescence, might increase the risk of potential side effects, mostly concerning opportunistic infections (18, 99, 114). The long-term effects on the immune system induced by DMTs and the potential additive effect on an immune system already showing features consistent with immunosenescence requires careful consideration of the individual characteristics to minimize potential detrimental effects of treatment.

In conclusion, the TCR repertoire investigation by HTS in healthy individuals has widened our understanding of how the adaptive immune system dynamics changes over lifetime. Furthermore, recent findings pointed out that immunosenescence impacts disease pathogenesis, including MS, and how patients respond to therapy. However, most of the studies do not focus on the relationship between aging and TCR dynamics, stressing for further insights. Deepening this investigation might be, in the future, an interesting tool for understanding disease mechanisms and customizing therapies to each individual patient.

AUTHOR CONTRIBUTIONS

RA conceived and wrote the manuscript. AM contributed in writing the manuscript. CB conceived and supervised the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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Clinical and Radiological Characteristics of Children and Adults With First-Attack Myelin Oligodendrocyte Glycoprotein Antibody Disease and Analysis of Risk Factors for Predicting the Severity at Disease Onset in Central China

*Yanfei Li, Haojie Xie, Jinwei Zhang, Yongyan Zhou, Lijun Jing, Yaobing Yao, Ranran Duan and Yanjie Jia**

Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

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Aurora Zanghì, Sant'Elia Hospital, Italy

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***Correspondence:**
Yanjie Jia
jiayanjie1971@zzu.edu.cn

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Objective: To analyze and compare different clinical, laboratory, and magnetic resonance imaging characteristics between pediatric and adult patients with first-attack myelin oligodendrocyte glycoprotein antibody disease (MOGAD) and to explore predictive factors for severity at disease onset.

Methods: Patients diagnosed with MOGAD at the First Affiliated Hospital of Zhengzhou University from January 2013 to August 2021 were enrolled in this retrospective study. Age at disease onset, sex, comorbidities, laboratory tests, magnetic resonance imaging (MRI) characteristics, and Expanded Disability Status Scale (EDSS) scores were collected and analyzed. The association between risk factors and initial EDSS scores at disease onset was analyzed using logistic regression models and Spearman correlation analyses. A receiver-operating characteristic (ROC) curve analysis was used to evaluate the predictive ability of the uric acid and homocysteine (Hcy) levels for the severity of neurological dysfunction at the onset of MOGAD.

Results: Sixty-seven patients (female, n=34; male, n=33) with first-attack MOGAD were included in this study. The mean age at onset was 26.43 ± 18.22 years (range: 3–79 years). Among patients <18 years of age, the most common presenting symptoms were loss of vision (36.0%), and nausea and vomiting (24.0%), and the most common disease spectrum was acute disseminated encephalomyelitis (ADEM) (40.0%). Among patients aged ≥18 years, the most common presenting symptoms were loss of vision (35.7%), paresthesia (33.3%), and paralysis (26.2%), and the most common disease spectrum was optic neuritis (35.7%). The most common lesions were cortical gray matter/paracortical white matter lesions in both pediatric and adult patients. Uric acid [odds ratio (OR)=1.014;

95% confidence interval (CI)=1.006–1.022; P=0.000] and serum Hcy (OR=1.125; 95% CI=1.017–1.246; P=0.023) levels were significantly associated with the severity of neurological dysfunction at disease onset. Uric acid levels (r=0.2583; P=0.035) and Hcy levels (r=0.3971; P=0.0009) were positively correlated with initial EDSS scores. The areas under the ROC curve were 0.7775 (95% CI= 0.6617–0.8933; P<0.001) and 0.6767 (95% CI=0.5433–0.8102, P=0.014) for uric acid and Hcy levels, respectively.

Conclusion: The clinical phenotype of MOGAD varies in patients of different ages. The most common disease spectrum was ADEM in patients aged<18 years, while optic neuritis was commonly found in patients aged ≥18 years. The uric acid and Hcy levels are risk factors for the severity of neurological dysfunction at disease onset in patients with first-attack MOGAD.

Keywords: myelin oligodendrocyte glycoprotein antibody disease, clinical and radiological characteristics, predictive factors, uric acid, homocysteine

INTRODUCTION

Myelin oligodendrocyte glycoprotein antibody disease (MOGAD) is a rare autoimmune disorder characterized by antibodies against the myelin oligodendrocyte glycoprotein (MOG) and has a wide spectrum of presenting clinical phenotypes. The majority of patients with MOGAD present with acute disseminated encephalomyelitis (ADEM), transverse myelitis (TM), recurrent optic neuritis (ON), neuromyelitis optica spectrum disorders (NMOSD), and multiple sclerosis (MS) (1, 2). Approximately 40% of patients with NMOSD who are seronegative for the aquaporin-4 (AQP4) antibody have MOGAD (3). Unlike AQP4, which is an astrocytic protein, MOG is localized on the outer layer of myelin and oligodendrocytes, which may induce oligodendrocyte and myelin injuries that result in inflammatory demyelination in the central nervous system (CNS) (4).

The clinical and radiological findings of MOGAD vary. Previous studies have focused on the clinical and radiological features or treatment and prognosis of patients with MOGAD (5–7); studies reporting detailed laboratory test data are rare. Although many cases have been reported in recent years, Chinese studies regarding MOGAD include relatively small sample sizes. Studies identifying reliable and sensitive markers to predict the severity of neurological impairment in patients with NMOSD and MS have been reported (8, 9); however, few studies have reported predictive factors for the severity of MOGAD at disease onset.

Clinical, laboratory, and magnetic resonance imaging (MRI) characteristics were analyzed, and predictive factors for the severity at disease onset of MOGAD were identified in this study. We focused on the first-attack MOGAD patients to rule out possible effects of previous treatments (such as glucocorticoids, immunoglobulin, and immunosuppressants) on laboratory results.

This is the first study to investigate predictive factors for the severity of MOGAD at disease onset, and the results will be useful for the early assessment of the prognosis of patients with MOGAD, allowing for more individualized treatment plans.

MATERIALS AND METHODS

Participants

Patients diagnosed with MOGAD at the First Affiliated Hospital of Zhengzhou University from January 2013 to August 2021 were retrospectively enrolled in this study. Patients who were seropositive for MOG antibodies on a live cell-based assay (CBA) with inflammatory attacks of the optic nerve, spinal cord, or brain were included (6).

The exclusion criteria were a non-first-attack of MOGAD, the coexistence of other diseases that may affect the Expanded Disability Status Scale (EDSS) scores, the use of corticosteroids or immunosuppressive therapies in the six months prior to admission, the use of drugs that may affect laboratory tests (including lipid-lowering drugs, homocysteine-lowering drugs, or hepatic or renal protectants), and the presence of hematological, infectious, or other diseases that may affect the laboratory tests or cerebrospinal fluid (CSF) analysis. The detailed selection process is shown in **Figure 1**.

This study was approved by the Ethics Committee of First Affiliated Hospital of Zhengzhou University (2019-KY-018) and was conducted according to the principles of the Declaration of Helsinki. All participants or their guardians provided written informed consent for their participation in the study.

Data Collection and Treatment

Age at onset, sex, comorbidities, clinical symptoms, treatments, laboratory tests [routine blood tests, liver function, renal function, lipids, thyroid hormones, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), homocysteine (Hcy), and antinuclear antibodies (ANAs)], CSF analysis (intracranial pressure, leukocyte count, and protein concentration), and imaging findings at admission were collected. The EDSS scores at admission, discharge, and follow-up were evaluated by an experienced neurologist. The clinical symptoms at admission were used to calculate the initial EDSS scores. Patients received different treatments according to their clinical symptoms and financial situation. Treatments including corticosteroids,

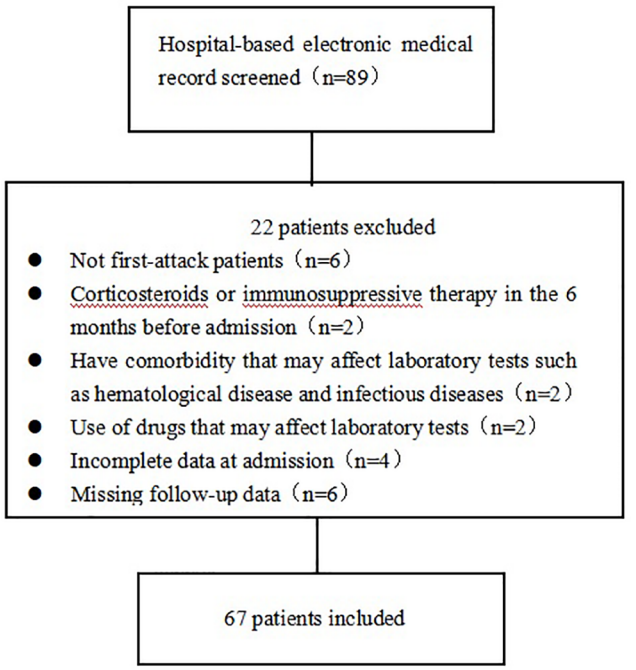

FIGURE 1 | Overview of the patient selection process.

immunoglobulins, and immunosuppressants (such as azathioprine, mycophenolate mofetil, and methotrexate) were recorded. Relapse was defined as new-onset or recurrent neurological symptoms lasting for at least 24 hours, resulting in an increase in the EDSS score of at least 0.5 points from the patient's lowest score. Relapse events occurring within 28 days of one another were considered a single relapse (10). Follow-up data were obtained *via* annual clinic visits or telephone interviews. The last follow-up date was November 15, 2021.

Blood samples were collected from patients after overnight fasting at 7:00–8:00 am the next day after admission. Blood and CSF samples were obtained prior to the administration of any treatments. MOG antibodies were measured by CBA. When MOG antibody titers were more than 1:10, we considered the MOG status positive. The MOG antibody titers of participants varied from 1:10 to 1:000++. Titers more than 1:000 were described as 1:000+ or 1:1000++. The tests were conducted in accordance with the manufacturer's protocols, and the examiners were blinded to the patients' diagnoses and clinical symptoms.

Imaging Data

MRI scans were performed using a 3.0T scanner (Philips Healthcare, Amsterdam, Netherlands). Sagittal T1-weighted images (T1WI), axial T1WI, T2-weighted images (T2WI), axial/sagittal fast fluid-attenuated inversion recovery (FLAIR) images, axial diffusion, apparent diffusion coefficient (ADC) mapped images, and contrast-enhanced axial, coronal, and sagittal T1WI images of the brain and sagittal T1WI, sagittal T2WI, axial T1WI, and axial T2WI images of the spine were analyzed. A gadolinium glutamine injection was used as the contrast agent. The locations of the lesions were recorded as deep white matter (WM), cortical gray matter/paracortical WM, periventricular WM, corpus callosum, basal ganglia, thalamus, midbrain, pons, medulla oblongata, cerebellum, cervical medulla, lumbar medulla, thoracic medulla, optic nerve, optic chiasma, and optic tract. All assessments were performed by two independent radiologists who were blinded to the patients' clinical features.

Grouping

The patients were divided into children (<18 years) and adults (≥18 years) based on the age at onset. The differences in clinical, laboratory, and radiological characteristics between children and adult patients were analyzed. The patients were then categorized based on initial EDSS score (≤3 or >3) to identify predictive factors for the severity of neurological dysfunction at disease onset (11).

Statistical Analyses

Continuous data with a normal distribution are presented as mean ± standard deviation. Continuous data with a non-normal distribution are presented as median ± interquartile range. Categorical variables are expressed as frequency (percentage, %). The differences between the two groups were analyzed using the Student's t-test and Wilcoxon test for normally and non-normally distributed data, respectively. Categorical data were compared using the chi-square test when comparing numbers ≥5 or Fisher's exact test when comparing small numbers <5. A univariate logistic regression analysis was used to identify potential predictive factors for the severity of neurological dysfunction at the onset of MOGAD. A multivariate logistic regression analysis was used to determine the independent predictive factors for the severity of neurological dysfunction at disease onset. Variables with a significance level of $P < 0.1$ in the univariate logistic regression analysis were included in the basic model. Variables clinically believed to have an impact on the initial EDSS (including age at onset and sex) and factors that affect Hcy levels (including folic acid and vitamin B12) were included in the adjusted model to analyze the stability of the associations between uric acid levels, Hcy levels, and the severity of neurological dysfunction at disease onset. A correlation analysis was performed using the Spearman correlation analysis. Receiver-operating characteristic (ROC) curve analyses were used to evaluate the diagnostic value of the uric acid and Hcy levels for the severity of neurological dysfunction at the onset of MOGAD.

All statistical analyses were performed using SPSS (version 26.0; IBM, Armonk, NY, USA). Statistical significance was set at $P < 0.05$.

RESULTS

Patient Demographics and Clinical Characteristics

Eighty-nine patients were diagnosed with MOGAD and were seropositive for MOG antibodies between January 2013 and August 2021 at First Affiliated Hospital of Zhengzhou

University. Sixty-seven (females, n=34; males, n=33) of these patients met the inclusion criteria and were included in this study.

The mean patient age at onset was 26.43 ± 18.22 years (range: 3–79 years). A total of 25 patients were <18 years of age (mean age: 9.12 ± 3.71 years; 15 females), and 42 patients were ≥18 years of age (mean age: 36.74 ± 15.32 years; 19 females). Sex was not significantly different between the groups (**Table 1**).

The most common clinical symptoms were loss of vision (35.8%), paresthesia (28.4%), and paralysis (23.9%), and the main disease spectrums included ON (35.8%) and TM (26.9%). Among patients <18 years old, the most common presenting symptoms were visual loss (36.0%) and nausea/vomiting (24.0%), and the main disease spectrum was ADEM (40.0%). Among patients ≥18 years old, the most common presenting symptoms were loss of vision (35.7%), paresthesia (33.3%), and paralysis (26.2%), and the most common disease spectrum was ON (35.7%). The incidences of nausea and vomiting (P=0.009), post polar syndrome (P=0.009), and ADEM (P=0.000) were significantly higher in patients <18 years old than in patients ≥18 years old.

The initial, discharge, and follow-up EDSS scores were not significantly different between the two groups (P>0.05). Most patients (94.0%) received corticosteroids, while 14 (20.9%) received intravenous immunoglobulins, and 8 (11.9%) received immunosuppressants. The treatments were not significantly different between the groups (P>0.05).

The median follow-up period was 7 months (range: 3–42 months). A total of 14 (20.9%) patients had a relapse during the study period. The median time between the first and second episodes was 5.5 months (range: 2–26 months). There were no significant differences in follow-up times (P=0.109) or relapse rates (P=0.63) between the two groups (**Table 2**).

Laboratory Examinations

Patients ≥18 years old had higher total bilirubin (P=0.001), creatinine (P=0.000), intracranial pressure (P=0.048), and CSF protein levels (P=0.000) than patients <18 years old (**Table 3**). Among all patients, the median intracranial pressure during lumbar puncture was normal, while the median CSF leukocyte count was elevated. Eight patients (11.9%) had positive CSF oligoclonal bands (OBs). Three patients (4.5%) had anti-N-methyl-D-aspartate receptor (anti-NMDAR) antibodies during the first attack of MOGAD (two patients presented with loss of vision and one patient with paralysis). Among the three patients, the disease spectrum was ON for two patients and myelitis for the other. The follow-up durations of the three patients were 20 months, 8 months, and 7 months, respectively. None of the three patients met the diagnostic criteria for anti-NMDAR autoimmune encephalitis or relapsed during the study period.

Radiological Characteristics

At disease onset, nine patients (13.4%) had normal brain or spinal cord MRIs, and then six patients presented abnormal MRI findings in subsequent disease processes. MOGAD lesions were identified in the optic nerve, cortical gray matter/paracortical WM, deep WM, periventricular WM, corpus callosum, basal ganglia, brainstem, cerebellum, and spinal cord. Overall, 41.8% of patients had lesions in the cortical gray matter/paracortical WM. The cortical gray matter/paracortical WM was the most common location for MOGAD lesions in both patients <18 years old and ≥18 years old. The cervical medulla was more involved than thoracic and lumbar lesions of the spinal cord (**Table 4**).

Predictive Factors for Disease Severity

There were no significant differences in age at onset, sex, erythrocyte count, hemoglobin, lymphocyte count, glucose, total protein, total bilirubin, creatinine, total cholesterol, triglycerides, high-density lipoprotein, ESR, CRP, free triiodothyronine (FT3), free thyroxine (FT4), thyroid-stimulating hormones (TSH), folic acid, vitamin B12, intracranial pressure, and CSF leukocyte count and protein concentration between the patients with an EDSS score >3 and those with an EDSS score ≤ 3 (**Table 5**). The proportions of positive ANAs (P=0.530) and positive thyroid peroxidase or

TABLE 1 | Patient demographics and comorbidities.

	All patients (n = 67)	Age<18 (n = 25)	Age≥18 (n = 42)	*P* value
Age at onset	26.43 ± 18.22	9.12 ± 3.71	36.74 ± 15.32	0.000*
Sex, female	34 (50.7)	15 (60.0)	19 (45.2)	0.242
Smoking	8 (11.9)	0	8 (19.4)	0.020*
Drinking	4 (6.0)	0	4 (9.5)	0.289
Hypertension	3 (4.5)	0	3 (7.1)	0.288
Diabetes	1 (1.5)	0	1 (2.4)	1.000
Coronary heart disease	3 (4.5)	0	3 (7.1)	0.288
Cerebrovascular disease	0	0	0	1.000
Anxiety/depression	2 (3.0)	0	2 (4.8)	0.525
Malignancy	1 (1.5)	0	1 (2.4)	1.000
Trauma	1 (1.5)	1 (4.0)	0	0.373
Autoimmune diseases				
Sjogren syndrome	1 (1.5)	0	1 (2.4)	1.000
Thyroid disease	5 (7.5)	2 (8.0)	3 (7.1)	1.000

Data are presented as number (percentage) or mean ± standard deviation.
**P < 0.05.*

TABLE 2 | Patient characteristics.

	All patients (n = 67)	Age<18 (n = 25)	Age≥18 (n = 42)	*P* value
Symptoms at onset				
Visual loss	24 (35.8)	9 (36.0)	15 (35.7)	0.981
Paralysis	16 (23.9)	5 (20.0)	11 (26.2)	0.636
Paresthesia	19 (28.4)	5 (20.0)	14 (33.3)	0.242
Nausea/Vomiting	7 (10.4)	6 (24.0)	1 (2.4)	0.009*
Fever	10 (14.9)	5 (20.0)	5 (11.9)	0.368
Headache	10 (14.9)	2 (8.0)	8 (19.0)	0.300
Dizziness	7 (10.4)	1(4.0)	6 (14.3)	0.244
Seizures	12 (17.9)	5 (20.0)	7 (16.7)	0.731
Speech disorders	2 (3.0)	1 (4.0)	1 (2.4)	1.000
Unsteady gait	5 (7.5)	4 (16.0)	1 (2.4)	0.061
Disturbance of consciousness	2 (3.0)	1 (4.0)	1 (2.4)	1.000
Disease spectrum				
Optic neuritis	24 (35.8)	9 (36.0)	15 (35.7)	0.981
Transverse myelitis	18 (26.9)	9 (36.0)	9 (21.4)	0.193
Acute disseminated encephalomyelitis	11(16.4)	10 (40.0)	1 (2.4)	0.000*
Brainstem syndrome	11 (16.4)	4 (16.0)	7 (16.7)	1.000
Post polar syndrome	7 (10.4)	6 (24.0)	1 (2.4)	0.009*
Treatment				
Corticosteroid	63 (94.0)	25 (100)	38 (90.5)	0.112
Intravenous immunoglobulin	14 (20.9)	7 (28.0)	7 (16.7)	0.270
Immunosuppressant				
Azathioprine	4 (6.0)	0	4 (9.5)	0.289
Mycophenolate mofetil	3 (4.5)	1 (4.0)	2 (4.8)	1.000
Methotrexate	1 (1.4)	1 (4.0)	0	0.373
Initial EDSS	4 (2–6)	4 (3–4.75)	4 (2–6)	0.974
Discharge EDSS	2 (1–4)	1.5 (1–3)	2 (1–4)	0.351
Follow-up EDSS	1.5 (1–2)	1 (1–2)	1.5 (1–2)	0.340
Follow-up interval (months)	7 (5–15)	6 (3.5–8.5)	8 (5.375–16.75)	0.109
Relapse	14 (20.9)	6 (24.0)	8 (19.0)	0.630

Data are presented as mean ± standard deviation, number (percentage), or median (interquartile range).
EDSS, expanded disability status scale scores.
**P < 0.05.*

thyroglobulin (P=0.454) antibodies were similar between the two groups. Patients with an EDSS score ≤3 had lower uric acid (P=0.000), low-density lipoprotein (P=0.013), and Hcy levels (P=0.015) than patients with an EDSS score >3.

Uric acid (odds ratio (OR)=1.014; 95% confidence interval (CI)=1.006–1.022; P=0.000) and serum Hcy levels (OR=1.125; 95% CI=1.017–1.246; P=0.023) were significantly correlated with the initial EDSS score (**Table 6**). In the basic model of multivariate logistic regression analysis, uric acid (OR=1.014; 95% CI=1.004–1.023; P=0.003) and Hcy levels (OR=1.125; 95% CI=1.002–1.262; P=0.045) were related with the severity of neurological dysfunction at the onset of MOGAD. In the adjusted model, uric acid (OR=1.019; 95% CI=1.007–1.031; P=0.002) and Hcy levels (OR=1.198; 95% CI=1.033–1.390; P=0.017) remained significantly correlated with the severity of neurological dysfunction at the onset of MOGAD (**Table 7**).

Uric acid levels (r=0.3905; P=0.0011) and Hcy levels (r=0.3971; P=0.0009) were found to be positively correlated with the initial EDSS scores (**Figure 2**).

ROC curve analysis was used to evaluate the predictive value of uric acid and Hcy for predicting the severity of neurological impairment at the onset of MOG. The areas under the ROC curve were 0.7775 (95% CI= 0.6617–0.8933; P<0.001) for uric acid levels and 0.6767 (95% CI=0.5433–0.8102, P=0.014) for Hcy levels. At a uric acid cut-off value of 223 μmol/L, the sensitivity for predicting an initial EDSS>3 was 64.29% and the specificity was 84.62%. At an Hcy cut-off value of 11.37 μmol/L, the sensitivity for predicting an initial EDSS>3 was 64.29% and the specificity was 79.49% (**Figure 3**).

DISCUSSION

MOGAD is an antibody-mediated inflammatory demyelinating disease of the CNS with a monophasic or relapsing course of neurological dysfunction. It presents as various phenotypes, such as ADEM, TM, recurrent ON, and cortical encephalitis (12, 13). In this study, we carried out a retrospective analysis to explore and compare different clinical, laboratory, and magnetic resonance imaging characteristics between children and adult patients with first-attack MOGAD, and explored risk factors for predicting the severity at disease onset of MOGAD. We found that the clinical phenotype of MOGAD varies in patients of different ages. The most common disease spectrum was ADEM in patients aged <18 years, while ON was commonly found in patients aged ≥18 years. The most common lesions were cortical

TABLE 3 | Laboratory data.

	All = patients (n = 67)	Age<18 (n = 25)	Age≥18 (n = 42)	*P* value
Leukocyte counts ($\times10^9$/L)	9563 ± 5.84	11.64 ± 8.26	8.36 ± 3.30	0.068
Erythrocyte counts ($\times10^{12}$/L)	4.39 ± 0.49	4.48 ± 0.41	4.33 ± 0.52	0.233
Hemoglobin (g/L)	128 (118.1–142.9)	124 (119.85–135)	132 (118–144.35)	0.218
Lymphocyte count ($\times10^9$/L)	1.96 ± 0.99	2.14 ± 1.19	1.86 ± 0.86	0.268
Glucose (mmol/L)	4.93 ± 1.12	5.02 ± 1.20	4.88 ± 1.09	0.612
Total protein (g/L)	66.63 ± 5.99	68.81 ± 4.77	65.33 ± 6.31	0.013*
Total bilirubin (μmol/L)	8.00 ± 4.65	6.0 ± 2.19	9.19 ± 5.30	0.001*
Uric acid (μmol/L)	257.61 ± 84.72	246.60 ± 100.54	264.17 ± 74.28	0.452
Creatinine (μmol/L)	54.51 ± 16.44	41.96 ± 15.96	61.98 ± 11.53	0.000*
Total cholesterol (mmol/L)	3.79 ± 0.96	3.57 ± 0.45	3.92 ± 1.10	0.114
Triglycerides (mmol/L)	1.23 ± 0.82	1.21 ± 0.66	1.24 ± 0.91	0.892
High-density lipoprotein (mmol/L)	1.19 ± 0.32	1.17 ± 0.25	1.20 ± 0.36	0.687
Low-density lipoprotein (mmol/L)	2.29 ± 0.72	2.07 ± 0.53	2.42 ± 0.80	0.055
ESR (mm/h)	10 (6–17)	11 (6–16.5)	8.65 (5.875–18.25)	0.484
CRP (mg/L)	1.82 (0.5–4.7)	1 (0.425–5.23)	2.705 (0.975–4.385)	0.248
FT3 (pmol/L)	4.88 ± 0.97	5.04 ± 1.15	4.78 ± 0.85	0.323
FT4 (pmol/L)	13.06 ± 2.86	13.59 ± 2.73	12.74 ± 2.92	0.243
TSH (pmol/L)	2.02 ± 1.35	1.68 ± 1.11	2.22 ± 1.45	0.114
Homocysteine levels (μmol/L)	14.74 ± 6.30	15.31 ± 6.56	14.40 ± 6.19	0.574
Folic acid (ng/mL)	7.59 ± 4.02	6.60 ± 3.21	8.19 ± 4.36	0.119
Vitamin B12 (pg/mL)	591.76 ± 397.33	614.66 ± 367.69	578.14 ± 417.72	0.719
ANAs positive	17 (25.4)	3 (12.0)	14 (33.3)	0.081
Thyroid peroxidase/Thyroglobulin antibodies positive	7 (10.4)	3 (12.0)	4 (9.5)	1.000
Intracranial pressure (mmH2O)	160 (135–180)	150 (135–170)	165 (140–200)	0.048*
CSF leukocyte counts ($\times10^6$/L)	12 (4–32)	15 (4.5–42)	11 (3.75–30.5)	0.266
CSF protein concentration (mg/L)	309.8 (233.2–388.9)	238 (183.55–306)	361.15 (294.525–446.875)	0.000*
Positive oligoclonal band	8 (11.9)	5 (20.0)	3 (7.1)	0.138
CSF anti-NMDAR(+)	3 (.4.5)	1 (4.0)	2 (4.8)	1.000
Serum AQP4-IgG(+)	0	0	0	–

Data are presented as mean ± standard deviation, number (percentage), or median (interquartile range).

NLR, neutrophil-to-lymphocyte ratio; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid-stimulating hormones; ANAs, antinuclear antibodies; CSF, cerebrospinal fluid; anti-NMDAR, anti-N-methyl-D-aspartate receptor; AQP4-IgG, aquaporin-4 immunoglobulins G.

**P < 0.05.*

gray matter/paracortical WM lesions in both pediatric and adult patients. Uric acid and Hcy levels were associated with the severity of neurological dysfunction at the disease onset of MOGAD. To the best of our knowledge, this is the first study to explore risk factors for predicting the severity of neurological dysfunction at the onset of MOGAD in a real-world setting. To eliminate the effects of previous treatments (such as glucocorticoids, immunoglobulin, and immunosuppressants)

TABLE 4 | Patients' radiological characteristics.

	All patients (n = 67)	Age<18 (n = 25)	Age≥18 (n = 42)	*P* value
Brain MRI				
Optic nerve	9 (13.4)	4 (160)	5 (11.9)	0.718
Cortical gray matter/paracortical WM	28 (41.8)	10 (40.0)	18 (42.9)	0819
Deep WM	9 (13.4)	3 (12.0)	6 (14.3)	1.000
Periventricular WM	15 (22.4)	6 (24)	9 (21.4)	0.807
Corpus callosum	2 (3.0)	1 (4.0)	1 (2.4)	1.000
Basal ganglia	7 (10.4)	4 (16.0)	3 (7.1)	0.411
Thalamus	10 (14.9)	3 (12.0)	7 (16.7)	0.732
Midbrain	5 (7.5)	2 (8.0)	3 (7.1)	1.000
Pons	14 (20.9)	6 (24.0)	8 (19.0)	0.630
Medulla oblongata	8 (11.9)	6 (24.0)	2 (4.8)	0.045*
Cerebellum	8 (11.9)	4 (16.0)	4 (9.5)	0.459
Spine MRI				
Cervical medulla	17 (25.4)	7 (28.0)	10 (23.8)	0.703
Thoracic medulla	16 (23.9)	6 (24.0)	10 (23.8)	0.986
Lumbar medulla	2 (3.0)	0	2 (4.8)	0.525

Data are presented as number (percentage).

MRI, magnetic resonance imaging; WM, white matter.

**P < 0.05.*

TABLE 5 | Laboratory data according to initial Expanded Disability Status Scale.

	EDSS ≤3 (n = 28)	EDSS>3 (n = 39)	*P* value
Age at onset, years	24.43 ± 16.52	27.87 ± 19.43	0.450
Sex, female	14 (50.0)	20 (51.3)	0.918
Leukocyte count ($\times10^9$/L)	10.91± 7.83	8.64 ± 3.68	0.118
Erythrocyte count ($\times10^{12}$/L)	4.39 ± 0.59	4.39 ± 0.41	0.950
Hemoglobin (g/L)	126.85 (116.5–145.775)	128 (122–142)	0.652
Lymphocyte count ($\times10^9$/L)	1.82 ± 0.73	2.07 ± 1.14	0.325
Glucose (mmol/L)	4.73 ± 1.00	5.08 ± 1.20	0.219
Total protein (g/L)	66.82 ± 6.30	66.49 ± 5.84	0.830
Total bilirubin (μmol/L)	7.41 ± 2.86	8.42 ± 5.59	0.335
Uric acid (μmol/L)	209.96 ± 77.03	291.82 ± 73.32	0.000*
Creatinine (μmol/L)	54.93 ± 16.62	54.21 ± 16.53	0.861
Total cholesterol (mmol/L)	3.61 ± 0.75	3.92 ± 1.08	0.188
Triglycerides (mmol/L)	1.13 ± 0.87	1.30 ± 0.78	0.404
High-density lipoprotein (mmol/L)	1.18 ± 0.33	1.19 ± 0.31	0.955
Low-density lipoprotein (mmol/L)	2.05 ± 0.50	2.46 ± 0.81	0.013*
ESR (mm/h)	8 (5.1–16.5)	10 (7–18)	0.184
CRP (mg/L)	1.585 (0.625–3.7725)	2.31 (0.5–5.14)	0.736
FT3 (pmol/L)	4.82 ± 0.83	4.92 ± 1.06	0.670
FT4 (pmol/L)	12.93 ± 2.60	13.15 ± 3.06	0.757
TSH (pmol/L)	1.85 ± 1.33	2.14 ± 1.37	0.389
Homocysteine levels (μmol/L)	12.56 ± 4.37	16.31 ± 7.02	0.015*
Folic acid (ng/mL)	7.69 ± 3.54	7.52 ± 4.38	0.867
Vitamin B12 (pg/mL)	487.83 ± 181.10	666.38 ± 486.52	0.069
ANAs positive	6 (15.4)	11 (28.2)	0.530
Thyroid peroxidase/Thyroglobulin antibodies positive	2 (5.1)	5 (12.8)	0.454
Intracranial pressure (mmH2O)	160 (126.25–180)	160 (140–175)	0.592
CSF leukocyte counts ($\times10^6$/L)	10 (4–27.75)	14 (4–40)	0.511
CSF protein concentration (mg/L)	309.6 (226.75–369.175)	309.8 (238–440.2)	0.457

Data are presented as mean ± standard deviation, number (percentage), or median (interquartile range).
NLR, neutrophil-to-lymphocyte ratio; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid-stimulating hormones; ANAs, antinuclear antibodies; CSF, cerebrospinal fluid; EDSS, Expanded Disability Status Scale.
**P < 0.05.*

on laboratory indicators, we focused on the first-attack NMOSD patients in the study.

Among 67 patients with first-attack MOGAD, the age of onset ranged from 3 to 79 years with an average age of 26.43 years, which was consistent with previous reports (14, 15). Prior evidence suggests that the female-to-male ratio among NMOSD patients is about 8:1 for AQP4-seropositive patients and 2:1 for AQP4-seronegative patients (16). Unlike the higher proportion of affected females seen in NMOSD, we found no sex differences between the affected males and females in patients with MOGAD, with a female-to-male ratio of 1.03:1. Similarly, there was no sex difference in either children or adult groups.

Loss of vision was the most common symptom in both children and adults in this study, and the most common disease spectrum was ADEM in children and ON in adults. A previous study reported that among patients <14 years of age with MOGAD, ADEM was the most frequent initial clinical symptom follow/ed by ON (17). In the study, we found that the incidence of nausea/vomiting and post polar syndrome in children was significantly higher than that in adults. However, when compared with children with AQP4-IgG positive NMOSD, patients with MOGAD tend to be less likely to present with post polar syndrome, but more likely to present with ADEM (18).

A previous study showed that 33.8–54% of children experience clinical relapses (19). Another report indicated that the relapse rate of MOG-Ab-positive patients aged ≥18 years were 44.8% and 61.8% after 2 and 5 years, respectively (20). Our study found that 24% of children patients experienced relapse, and adult patients showed a relapse rate of 19%, which was lower than previously-reported rates. This difference may be due to the different mean follow-up intervals of the studies.

Most patients with MOGAD present normal intracranial pressure and CSF protein concentration. Median CSF leukocyte counts of MOGAD were slightly elevated in our study. A previous study indicated that positive OBs were more commonly found in children and occurred in 6–17% of MOGAD patients. In the present study, the incidence of OBs was 11.9%, consistent with a previous study (21). A cohort study enrolled 42 patients with MOGAD and 491 patients with NMOSD and found that 11.9% patients with MOGAD and 0.6% patients with NMOSD had overlapping anti-NMDAR encephalitis (22).Three patients (4.4%) with MOGAD were found to have anti-NMDAR antibodies at first attack in our study. A previous study found that patients with MOGAD with positive anti-NMDAR antibodies had a higher relapse compared with patients with anti-NMDAR encephalitis (17). We speculate the

TABLE 6 | Univariate logistic regression analysis of potential predictive factors for the severity at the onset of myelin oligodendrocyte glycoprotein antibody disease.

Variables	Univariate analysis OR (95% CI)	*P* value
Age at onset	1.011 (0.983–1.039)	0.444
Sex	0.950 (0.360–2.509)	0.918
Leukocyte count	0.920 (0.819–1.034)	0.163
Erythrocyte count	0.966 (0.353–2.646)	0.946
Hemoglobin	1.005 (0.984–1.027)	0.638
Lymphocyte count	1.301 (0.772–2.194)	0.323
Glucose	1.338 (0.840–2.130)	0.220
Total protein	0.991 (0.913–1.075)	0.826
Total bilirubin	1.053 (0.938–1.181)	0.382
Uric acid	1.014 (1.006–1.022)	0.000*
Creatinine	0.997 (0.968–1.027)	0.858
Total cholesterol	1.437 (0.835–2.473)	0.191
Triglycerides	1.310 (0.698-2.456)	0.401
High-density lipoprotein	1.045 (0.226–4.830)	0.955
Low-density lipoprotein	2.496 (1.108–5.623)	0.027*
ESR	1.029 (0.983–1.077)	0.216
CRP	0.978 (0.932–1.027)	0.372
FT3	1.120 (0.671–1.867)	0.665
FT4	1.028 (0.866–1.221)	0.753
TSH	1.183 (0.810–1.728)	0.385
Homocysteine levels	1.125 (1.017–1.246)	0.023*
Folic acid	0.989 (0.876–1.117)	0.864
Vitamin B12	1.001 (1.000–1.003)	0.086
ANAs positive	0.694 (0.222–2.172)	0.531
Thyroid peroxidase/Thyroglobulin antibodies positive	0.523 (0.094–2.914)	0.460
Intracranial pressure	1.006 (0.992–1.020)	0.400
CSF leukocyte count	1.003 (0.990–1.015)	0.670
CSF protein concentration	1.002 (0.999–1.006)	0.223

NLR, neutrophil-to-lymphocyte ratio; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid stimulating hormones; ANAs, antinuclear antibodies; CSF, cerebrospinal fluid; OR, odds ratio; CI, confidence interval.
**P < 0.05.*

possible reason may be MOGAD causes oligodendrocyte damage and primary demyelination. N-acetyl aspartate (NAA) is mainly catabolised in oligodendrocytes. Defective NAA metabolism in oligodendrocytes may lead to increased NAA, which is a sign of acute neuronal damage (23). In the study, during follow-up, none of the three patients relapsed. Longer follow-up periods are required to further investigate the relapse rate in patients with MOGAD with anti-NMDAR antibodies.

Our study found that the most common lesions in children and adults were cortical gray matter/paracortical WM, which were present in 41.8% of all patients with MOGAD. Previous reports showed that in comparison with AQP4-positive NMOSD patients, MOGAD patients are more likely to have cortical gray matter/paracortical WM region involvement. Salama et al. reported that cortical gray matter/paracortical WM lesions on brain MRI might help distinguish MOGAD from AQP4-positive

TABLE 7 | Multivariate logistic regression analysis of predictive factors for the severity at the onset of myelin oligodendrocyte glycoprotein antibody disease.

Variables	Multivariate analysis			
	[a]Basic Model		[b]Adjusted model	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age			1.014 (0.976–1.054)	0.475
Sex, female			0.262 (0.051–1.339)	0.108
Uric acid	1.014 (1.004–1.023)	0.003*	1.019 (1.007–1.031)	0.002*
Low-density lipoprotein	2.938 (1.068–8.088)	0.037*	2.298 (0.818–6.456)	0.114
Homocysteine levels	1.125 (1.002–1.262)	0.045*	1.198 (1.033–1.390)	0.017*
Folic acid			1.003 (0.829–1.214)	0.973
Vitamin B12	1.002 (0.999–1.005)	0.140	1.002 (0.999–1.005)	0.174

[a]*Basic Model: Variables with P < 0.1 in the univariate logistic regression analysis were included in the multivariate model.*
[b]*Adjusted model: Variables with P < 0.1 in the univariate logistic regression analysis or variables clinically believed to have an impact on the initial Expanded Disability Status Scale (including age at onset and sex) and factors that affect Hcy levels (including folic acid and vitamin B12) were included in the adjusted model.*
**P < 0.05.*
CI, confidence interval; OR, odds ratio.

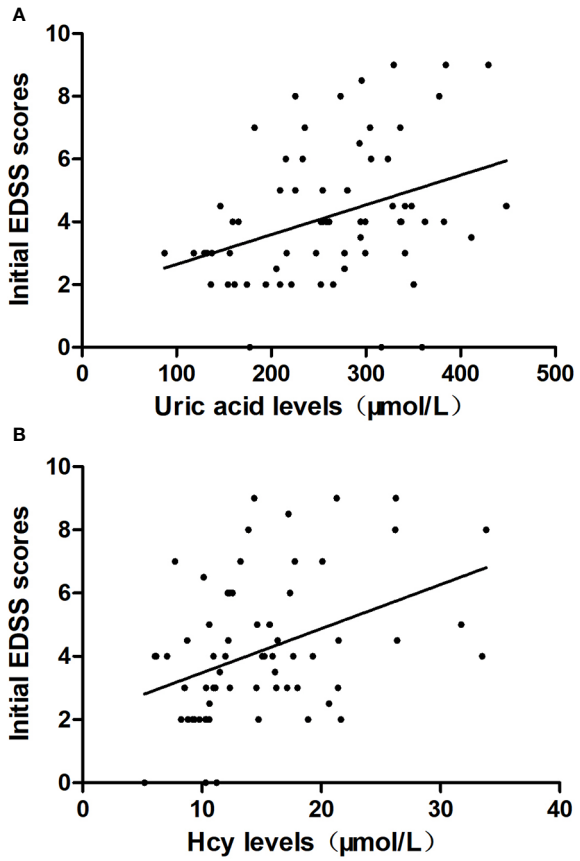

FIGURE 2 | The correlation between uric acid levels, homocysteine (Hcy) levels and the initial Expanded Disability Status Scale (EDSS) scores. **(A)** The correlation between uric acid levels and the initial EDSS scores is shown. **(B)** The correlation between Hcy levels and the initial EDSS scores is shown.

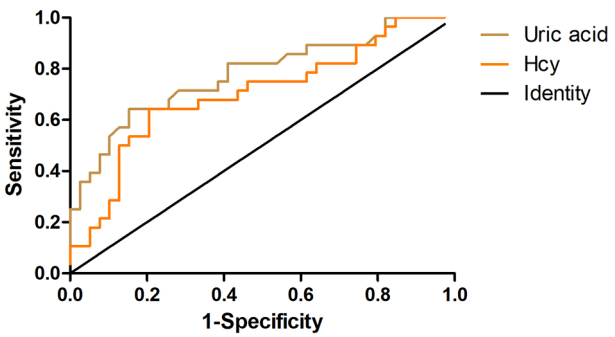

FIGURE 3 | The receiver operating characteristic (ROC) curves. The ROC curves show the predictive abilities of uric acid (light brown) and serum homocysteine (orange) for the severity of neurological dysfunction at the onset of myelin oligodendrocyte glycoprotein antibody disease (MOGAD).

NMOSD (6). Thus, we speculated that cortical gray matter/paracortical WM might act as a potential imaging marker for MOGAD. Thalamic and pontine lesions have also been reported as more common in patients with MOGAD than in patients with AQP4-positive NMOSD (20). Bilateral thalamic lesions at onset have been reported in approximately 60% of children with MOGAD (15). We found that brainstem involvement, especially of the pons and medulla oblongata, was commonly found in children, whereas thalamic lesions were less common compared with a previous report (15). It has been reported that >50% of patients with MOGAD have hyperintense lesions in the spinal cord (especially in the cervical or thoracic regions) on T2 images (14). In the study, the incidence of spinal cord lesions was 52% in children and 52.4% in adults, respectively, with cervical or thoracic segments predominantly involved.

In this study, patients with an initial EDSS score ≤3 had lower levels of uric acid, low-density lipoproteins, and Hcy than patients with an initial EDSS score >3. Uric acid and Hcy levels were correlated with the severity of neurological dysfunction at the onset of MOGAD and with the initial EDSS score. The optimal cut-off values of uric acid and Hcy levels for predicting the severity of neurological dysfunction at the onset of MOGAD were 223μmol/L and 11.37 μmol/L, respectively.

Uric acid is a natural product of the adenine nucleotide metabolic pathway, and its role in the CNS remains unclear. Previous studies have reported that uric acid is a strong free radical scavenger and antioxidant, while other studies have shown that uric acid reflects the production of free radicals by xanthine oxidase and is related to the glutamate-mediated excitotoxicity in neurological patients, which reflects the rate of adenosine triphosphate (ATP) catabolism (24, 25). In the attempt to find out reliable diagnostic or prognostic biomarkers, uric acid in biological fluids (plasma/serum, CSF, and urine) was measured in patients with MS or NMOSD. The uric acid levels in the biological fluids of patients with MS or NMOSD remain controversial. Some previous studies observed decreased uric acid levels in both serum/CSF of MS and NMOSD patients. Researchers have speculated that MS/NMOSD patients with low uric acid levels were unable to inhibit free radical toxicity and inflammation occurring in diseases. To deplete the excessively produced free radicals, the consumption of uric acid increases with lower uric acid levels as a result (26, 27). Contrarily, other scientists have observed different results. Amorini et al. demonstrated that uric acid levels were significantly higher in both the CSF and serum of MS patients in comparison with control groups, leading to the hypothesis that uric acid does not act as an antioxidant but indicates ongoing accelerated purine catabolism, possibly secondary to energy imbalance in MS (28). Another study reported significantly increased uric acid levels in the CSF and mildly increased uric acid levels in the serum of patients with NMOSD during relapse compared to control patients. Patients with an EDSS score >3.5 were reported to have higher uric acid levels in the CSF than patients with an EDSS score ≤

3.5 (29). In the present study, we demonstrated that uric acid levels were related to the severity of MOGAD. Further studies are needed to identify the possible mechanisms of uric acid in MOGAD.

Serum Hcy levels were found to be associated with the prognosis of MS and NMOSD (30, 31). Elevated Hcy levels could cause oxidative stress, mitochondrial dysfunction, myelin sheath degeneration, and apoptosis (32–34). In our previous study, we found that Hcy was an independent predictor of relapse and poor prognosis in first-attack NMOSD patients (31). In the present study, we found that serum Hcy levels were higher in patients with EDSS >3 than in patients with EDSS ≤3 and positively correlated with the severity of neurological dysfunction at onset of MOGAD.

This study had some limitations. First, the sample size was relatively small, and the patients were from a single center. Second, the follow-up period was relatively short. Finally, the antibody titer results of some patients were not recorded. Therefore, the results of this study require further validation in larger multicenter studies with longer follow-up periods.

In conclusion, the results of this study suggest that MOGAD presents differently in patients of different ages. Uric acid and Hcy levels may be useful predictors of the severity of MOGAD at onset during the first attack, and elevated uric acid and Hcy levels are associated with severe neurological disabilities. These results indicate that more aggressive therapies should be administered when these predictors are observed. Further studies are needed to validate these conclusions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of First Affiliated Hospital of Zhengzhou University. Written informed consent to participate in this study was provided by the participants' legal guardian/ next of kin.

AUTHOR CONTRIBUTIONS

YL: Methodology, Formal analysis, Data curation, Writing—original draft, Writing—review and editing. HX: Investigation, Writing—review and editing. JZ: Investigation, Writing—review and editing. YZ: Methodology, Investigation, Writing—review and editing. LJ: Data curation. YY: Formal analysis. RD: Formal analysis. YJ: Conceptualization, Methodology, Supervision, Funding acquisition. The first draft of the manuscript was written by YL and all authors commented on previous versions of the manuscript. All authors contributed to the article and approved the submitted version.

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Integrated Lymphopenia Analysis in Younger and Older Patients With Multiple Sclerosis Treated With Cladribine Tablets

Gavin Giovannoni[1], Patricia K. Coyle[2], Patrick Vermersch[3], Bryan Walker[4], Julie Aldridge[5], Axel Nolting[6], Andrew Galazka[7], Caroline Lemieux[8] and Thomas P. Leist[9]*

[1] Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom, [2] Department of Neurology, Stony Brook University, Stony Brook, NY, United States, [3] Univ. Lille, INSERM U1172, Lille Neurosciences and Cognition, CHU Lille, FHU Precise, Lille, France, [4] Department of Neurology, Duke University School of Medicine, Durham, NC, United States, [5] Research and Development Global Biostatistics, EMD Serono Research & Development Institute, Inc. (an affiliate of Merck KGaA), Billerica, MA, United States, [6] Global Patient Safety, Merck Healthcare KGaA, Darmstadt, Germany, [7] Global Clinical Development, Ares Trading SA (an affiliate of Merck KGaA), Eysins, Switzerland, [8] North American Medical Affairs, EMD Inc. (an affiliate of Merck KGaA), Mississauga, ON, Canada, [9] Comprehensive Multiple Sclerosis Center, Jefferson University, Philadelphia, PA, United States

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Christian Messina, Gaspare Rodolico Hospital, Italy

***Correspondence:**
Gavin Giovannoni
g.giovannoni@qmul.ac.uk

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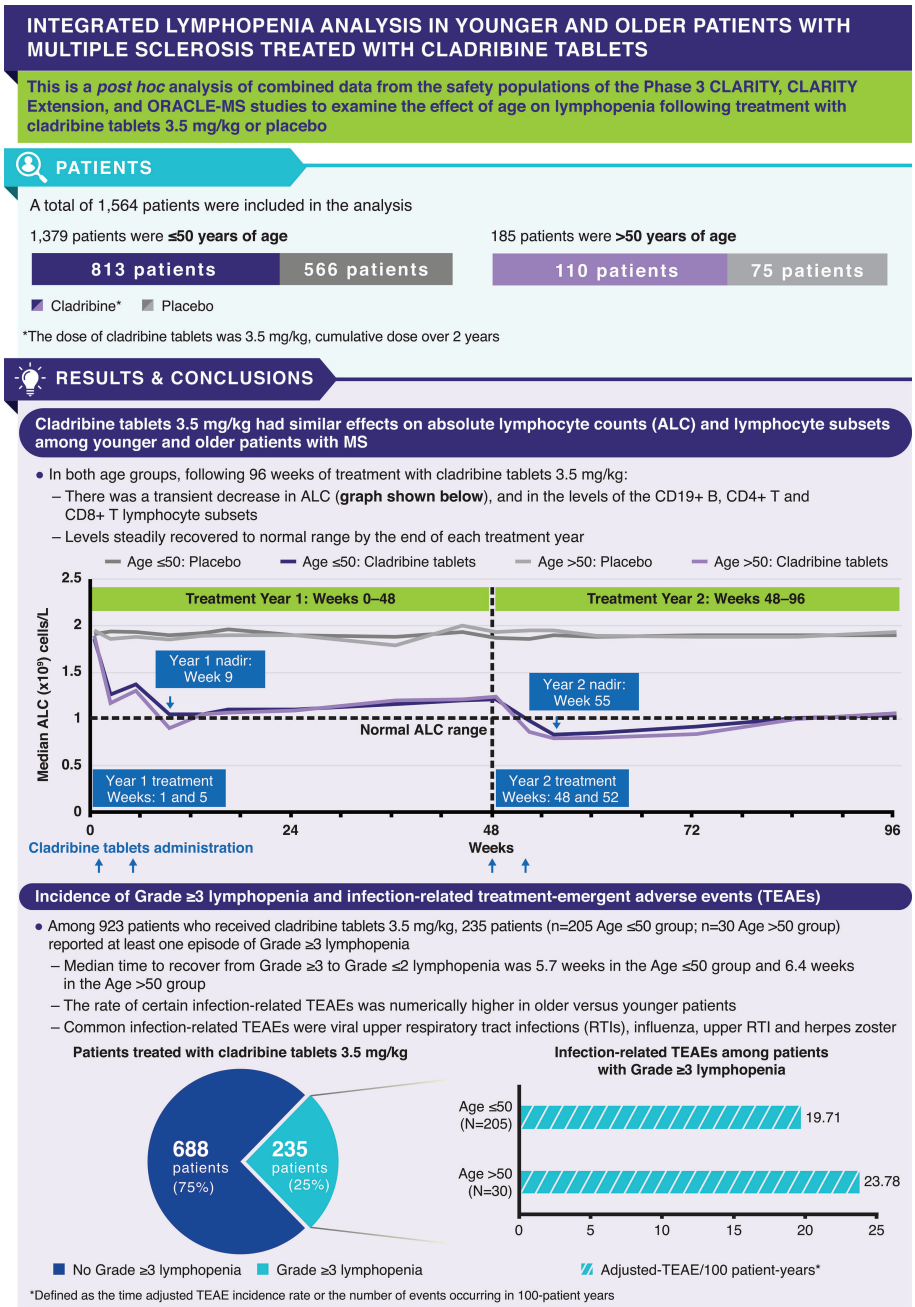
Cladribine tablets (CladT) preferentially reduce B and T lymphocyte levels. As aging is associated with a decline in immune function, the effect of CladT on lymphocyte levels may differ by age. This *post hoc* analysis combined data from the Phase 3 CLARITY, CLARITY Extension, and ORACLE-MS studies to examine the effect of age (≤50 or >50 years) on lymphopenia following CladT 3.5 mg/kg (CladT3.5; cumulative dose over 2 years) treatment over 96 weeks. Both CladT3.5 and placebo were given over Weeks 1 and 5 (Year 1 treatment) and Weeks 48 and 52 (Year 2 treatment) from the start of the studies. Absolute lymphocyte count (ALC) and levels of lymphocyte subsets were examined in 1564 patients (Age ≤50 [placebo: N=566; CladT3.5: N=813]; Age >50 [placebo: N=75; CladT3.5: N=110]). In both age groups, following CladT3.5 treatment, nadir for ALC occurred at Week 9 (8 weeks following start of Year 1 treatment) and Week 55 (7 weeks following start of Year 2 treatment) of the 96-week period; for CD19+ B lymphocytes, nadir occurred at Week 9 (Year 1) and Week 52 (Year 2). For CD4+ T lymphocytes, nadir occurred at Week 16 (Year 1) in both age groups, and at Weeks 60 and 72 (Year 2) in the Age ≤50 and >50 groups, respectively. Nadir for CD8+ T lymphocytes occurred at Week 16 (Year 1) and Week 72 (Year 2) in the Age ≤50 group and levels remained in the normal range; nadir occurred at Week 9 (Year 1) and Week 96 (Year 2) in the Age >50 group. Lymphocyte recovery began soon after nadir following CladT3.5 treatment and median levels reached normal range by end of the treatment year in both age groups. By Week 96, ~25% of patients treated with CladT3.5 reported ≥1 episode of Grade ≥3 lymphopenia (Gr≥3L). The rate of certain infections was numerically higher in older versus younger patients who experienced Gr≥3L. In conclusion, CladT3.5 had a similar effect on ALC and lymphocyte subsets in both younger and older patient groups.

Keywords: cladribine tablets, lymphocyte subsets, lymphopenia, multiple sclerosis, age



GRAPHICAL ABSTRACT |

INTRODUCTION

Cladribine tablets are an oral treatment for adults with relapsing forms of multiple sclerosis (MS), which was approved by the European Medicines Agency in 2017 and the US Food and Drug Administration in 2019, and has now received worldwide approval in over 80 countries (1–3). Cladribine treatment is a therapy that induces transient lymphopenia when administered over a very short period, followed by longer treatment-free periods (2, 4, 5). The approved dose of cladribine tablets 10 mg (3.5 mg/kg cumulative dose over 2 years; referred to here as cladribine tablets 3.5 mg/kg) has demonstrated efficacy in patients with relapsing forms of MS, including relapsing-remitting MS (RRMS) (6–10). Cladribine (2-chlorodeoxyadenosine [2-CdA]) is a synthetic, deoxyadenosine analog; it is a prodrug that is preferentially activated in lymphocytes due to their constitutively high deoxycytidine kinase and relatively low 5'-nucleotidase levels (3). Cladribine selectively reduces circulating T and B lymphocytes,

which play a central role in the pathogenesis of MS (2, 11). Consistent with the mechanism of action of cladribine, lymphopenia (Grade ≥1) has been reported in ~90% of patients treated with cladribine tablets 3.5 mg/kg over 2 years (7). Analysis of pooled data from the Phase 3 CLARITY and CLARITY Extension trials and the PREMIERE registry showed that treatment with cladribine tablets 3.5 mg/kg resulted in transient decreases in absolute lymphocyte counts (ALC), associated with a reduction in the number of CD19+ B, CD4+ T and CD8+ T lymphocyte subsets (4). This was followed by a recovery phase within weeks of nadir, wherein lymphocyte levels gradually returned to normal levels (4).

The incidence and prevalence of MS have increased over the past decade, with the peak prevalence shifting from the age of 40 years to ~60 years, due to improvements in MS diagnosis and life-expectancy (12, 13). Biological aging of the immune system, known as immunosenescence, is influenced by both genetic and environmental factors and is associated with a reduced ability to fight infections and develop immunological memory (14–17). As immunosenescence occurs with age, total lymphocyte levels are generally lower in older people compared to younger people, therefore use of disease-modifying therapies (DMTs) that further reduce lymphocyte function could potentially put older patients with MS at greater risk of adverse events (12, 15, 18). In addition, age is an important modifier of DMT efficacy; for some DMTs, slowing of MS disability progression decreases with increasing patient age (19). Opportunistic infections such as cryptococcal meningitis are common with some DMTs and this risk increases with age (12). The risk of varicella zoster virus (VZV) reactivation is higher among the elderly (20), and use of DMTs may further increase the risk of viral reactivation among older patients with MS. Low lymphocyte levels, especially in the central nervous system (CNS), are also associated with an increased risk for progressive multifocal leukoencephalopathy (PML) and aging appears to contribute to this risk (18). Given these observations, it is important to understand the immunological impact of DMTs on older versus younger patients with MS.

This *post hoc* analysis aimed to further explore and characterize the impact of age (≤50 years *vs.* >50 years) on the nature of lymphopenia experienced by patients treated with cladribine tablets 3.5 mg/kg in an integrated safety analysis.

METHODS

Trial Design

CLARITY (NCT00213135) and ORACLE-MS (NCT00725985) were Phase 3, double-blind, randomized, placebo-controlled, 96-week studies of cladribine tablets in patients with RRMS and a first clinical demyelination event, respectively (7, 21). Details have been published previously, but to briefly summarize, both studies evaluated the efficacy and safety of cladribine tablets 3.5 or 5.25 mg/kg (cumulative dose over 2 years) versus placebo in previously treated or untreated patients (7, 21). For the 3.5 mg/kg dose regimen, cladribine tablets (10 mg tablets) were administered over 2 weeks at 0.875 mg/kg/week for 4–5 consecutive days starting on Day 1 of Weeks 1 and 5 of Year 1; this was followed by two further treatment weeks in Year 2 (at Weeks 48 and 52 from the start of study). CLARITY Extension (NCT00641537) was a double-blind, 120-week study that investigated long-term safety and efficacy of cladribine tablets 3.5 mg/kg versus placebo in eligible patients who completed CLARITY. Patients who received placebo in CLARITY were assigned to cladribine tablets 3.5 mg/kg, while those treated with cladribine tablets were re-randomized (2:1) to an additional 2-year course of cladribine tablets 3.5 mg/kg or placebo (9).

Post Hoc Analysis

This *post hoc* analysis was conducted to evaluate the effect of age (Age ≥18–≤50 years [Age ≤50 group] and Age >50–≤65 years [Age >50 group]) at baseline on ALC and levels of lymphocyte subsets (CD19+ B, CD4+ T and CD8+ T lymphocytes) among patients treated with placebo or cladribine tablets 3.5 mg/kg. The analysis period was between Weeks 0–96 of treatment with cladribine tablets 3.5 mg/kg in ORACLE-MS, CLARITY, and CLARITY Extension (treatment Year 1: Weeks 0–48; treatment Year 2: Weeks 48–96). Of the CLARITY patients who entered CLARITY Extension, only patients who received placebo in CLARITY and cladribine tablets 3.5 mg/kg in CLARITY Extension and those who received cladribine tablets 3.5 mg/kg in CLARITY and placebo in CLARITY Extension were included in this analysis. The analysis population was the cladribine tablets 3.5 mg/kg monotherapy oral cohort (4, 7, 9, 21). Assessments included incidence of Grade ≥3 lymphopenia (Gr≥3L); Gr≥3L was defined as ALC levels <500 cells/μL based on the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 3.0 toxicity grading system. The time to recovery from first Gr≥3L to Grade ≤2 lymphopenia (ALC ≥500 cells/μL) was also assessed. All assessments were performed for each age group (≤50 years and >50 years). Among patients who had an episode of Gr≥3L, the rate and type of treatment-emergent adverse events (TEAEs) of viral and bacterial infections was evaluated. The incidence of TEAEs were summarized by Preferred Term (PT) and severity, coded according to the MedDRA dictionary Version 20.0 and expressed in adjusted-TEAE/100 patient-years (Adj-TEAE/100PY). Adj-TEAE/100PY is the time-adjusted incidence rate of TEAEs which can be interpreted as the number of events occurring in 100PY; the confidence interval (CI) is computed with the Wald method for the number of patients with events using a Poisson regression model with fixed effect for treatment group and with log of time at risk as an offset. When the observed rate is zero, the lower limit is set to zero and the exact formula is used for the upper limit. All analyses were performed using Statistical Analysis Software (SAS®), Version 9.4 or higher.

RESULTS

Patients

A total of 1564 patients were included in this *post hoc* analysis. Of these, 1379 were ≥18 to ≤50 years (Age ≤50 group [placebo: N=566; cladribine tablets 3.5 mg/kg: N=813]) and 185 were >50 to ≤65 years (Age >50 group [placebo: N=75; cladribine tablets 3.5 mg/kg: N=110]) of age. The mean age in the younger age group

was 35.1–35.2 years and in the older age group was 53.7–54.0 years (**Table 1**). Baseline demographics and disease characteristics were generally well balanced between age groups. Most patients (79.5–80.9%) were treatment naïve at baseline in both age groups. The two age groups also had broadly similar baseline median ALC (1.86–1.95 x 10^9 cells/L; **Table 2**) and lymphocyte subset levels (CD19+ B cells: 204–225 cells/μL; CD4+ T cells: 758–952 cells/μL; CD8+ T cells: 338–409 cell/μL; **Supplementary Tables 1**–**3**). Compared with the Age ≤50 group (both placebo- and cladribine tablets-treated patients), the Age >50 group had a higher proportion of women (73.6–76.0% *vs.* 64.8–65.3%), longer disease duration (median 11.4–12.7 years *vs.* 6.4–6.6 years), and more patients with at least one relapse at baseline or in the 12 months prior to study entry (66.3–90.7% *vs.* 53.0–64.5%; **Table 1**). Furthermore, the older age group had a slightly higher proportion of patients with ≥9 T2 lesions (84.9–94.5% *vs.* 85.8–87.2%), but a lower proportion with ≥1 T1 Gd+ lesions (13.6–16.2% *vs.* 33.3–35.5%) at baseline.

Changes in ALC During 2 Years of Active Treatment With Cladribine Tablets 3.5 mg/kg

In both age groups, the Year 1 (Weeks 0–48) ALC nadir in patients treated with cladribine tablets 3.5 mg/kg occurred at Week 9 and Year 2 (Weeks 48–96) nadir occurred at Week 55 (7 weeks following start of Year 2 dosing; **Figure 1A**). The median (interquartile range [IQR]) ALC at Week 9 for the Age ≤50 and >50 groups was 1.05 (0.80, 1.30) x10^9 cells/L and 0.9 (0.72, 1.29) x10^9 cells/L, respectively; median ALC recovered to normal range (above the lower limit of normal [LLN] of 1.02 x10^9 cells/L) by end of Year 1 (Week 48; **Table 2**). The median (IQR) for the Age ≤50 and >50 groups at Week 55 were below LLN at 0.83 (0.60, 1.08) x10^9 cells/L and 0.79 (0.57, 1.0) x10^9 cells/L, respectively. This was followed by a gradual ALC recovery to normal range at the end of Year 2 (Week 96; **Figure 1A**).

Effects of Cladribine Tablets 3.5 mg/kg on Lymphocyte Subsets

In both study years, a decrease in the levels of CD19+ B, CD4+ T and CD8+ T lymphocytes was observed with cladribine tablets 3.5 mg/kg treatment in both age groups; median lymphocyte levels recovered to normal range by the end of each study year.

CD19+ B Lymphocytes

For both age groups, following treatment with cladribine tablets 3.5 mg/kg, the Year 1 nadir for CD19+ B lymphocyte levels occurred at Week 9 and was below LLN; median (IQR) for

TABLE 1 | Baseline demographics and disease characteristics of patients in the ≤50 and >50 years age groups.

	Age ≤50 years		Age >50 years	
	Placebo (N=566)	CladT3.5 (N=813)	Placebo (N=75)	CladT3.5 (N=110)
Age, years, mean (SD)				
Mean (SD)	34.9 (8.0)	34.7 (8.4)	54.3 (3.1)	54.6 (3.7)
Median (range)	35.1 (18–50)	35.2 (18–50)	54.0 (50–64)	53.7 (50–65)
Female, n (%)	367 (64.8)	531 (65.3)	57 (76.0)	81 (73.6)
Disease duration, years, median (range)	6.4 (0.4–31.3)[a]	6.6 (0.3–32.8)[b]	12.7 (0.5–39.5)[c]	11.4 (0.4–42.3)[d]
<3 years, n (%)	90 (24.5)	125 (21.5)	7 (10.3)	8 (7.8)
3–10 years, n (%)	172 (46.9)	291 (50.0)	22 (32.4)	34 (33.0)
>10 years, n (%)	105 (28.6)	166 (28.5)	39 (57.4)	61 (59.2)
Prior use of DMT				
No DMTs	450 (79.5)	650 (80.0)	60 (80.0)	89 (80.9)
1 DMT	91 (16.1)	128 (15.7)	11 (14.7)	16 (14.5)
≥2 DMTs	25 (4.4)	35 (4.3)	4 (5.3)	5 (4.5)
Number of relapses at baseline (12 months prior to study entry), n (%)				
0	201 (35.5)	382 (47.0)	7 (9.3)	37 (33.6)
1	254 (44.9)	305 (37.5)	52 (69.3)	58 (52.7)
≥2	111 (19.6)	126 (15.5)	16 (21.3)	15 (13.6)
EDSS at baseline, median (range)	2.0 (0–5.5)	2.0 (0–6.5)	3.5 (1.0–5.5)	3.5 (0–6.5)
Number of T1 Gd+ lesions at baseline, mean (SD)	0.9 (2.3)[e]	1.2 (3.4)[f]	0.3 (0.9)[g]	0.3 (1.0)
No lesions, n (%)	377 (66.7)	524 (64.5)	62 (83.8)	95 (86.4)
≥1 lesion, n (%)	188 (33.3)	288 (35.5)	12 (16.2)	15 (13.6)
Number of T2 lesions at baseline, mean (SD)	27.7 (22.0)[e]	30.1 (22.3)[f]	21.9 (14.0)[g]	26.2 (13.3)
<9 T2 lesions, n (%)	80 (14.2)	104 (12.8)	11 (15.1)	6 (5.5)
≥9 T2 lesions, n (%)	485 (85.8)	708 (87.2)	62 (84.9)	104 (94.5)
T2 lesion volume (cm^3), mean (SD)	10.6 (12.2)[e]	11.9 (14.0)[f]	12.8 (12.2)[g]	16.3 (18.8)

[a]n=367, [b]n=582, [c]n=68, [d]n=103, [e]n=565, [f]n=812, [g]n=74.

CladT3.5, cladribine tablets 3.5 mg/kg, cumulative dose over 2 years; DMT, disease-modifying therapy; EDSS, Expanded Disability Status Scale; Gd+, gadolinium enhancing; SD, standard deviation.

TABLE 2 | Nadir and ALC recovery in years 1 and 2 by age group.

ALC ($x10^9$/L), median (IQR)	Age ≤50 years		Age >50 years	
	Placebo (N=566)	CladT3.5 (N=813)	Placebo (N=75)	CladT3.5 (N=110)
Baseline				
n	565	811	75	110
ALC	1.91	1.86	1.95	1.89
	(1.57, 2.33)	(1.52, 2.29)	(1.50, 2.31)	(1.53, 2.41)
Year 1				
Week 9[a,c], n	535	766	72	107
ALC	1.90	1.05	1.85	0.90
	(1.56, 2.29)	(0.80, 1.30)	(1.48, 2.27)	(0.72, 1.29)
Week 48[b,c], n	351	511	44	76
ALC	1.87	1.21	1.93	1.24
	(1.54, 2.25)	(0.95, 1.50)	(1.51, 2.36)	(1.0, 1.52)
Year 2				
Week 55[a,c], n	251	478	29	60
ALC	1.90	0.83	1.95	0.79
	(1.56, 2.33)	(0.60, 1.08)	(1.59, 2.50)	(0.57, 1.0)
Week 96[b,c], n	379	573	60	96
ALC	1.90	1.04	1.93	1.06
	(1.54, 2.27)	(0.80, 1.34)	(1.46, 2.41)	(0.85, 1.29)

Lower limit of normal = 1.02 $x10^9$/L.

[a]*Nadir for ALC in patients treated with CladT3.5.*

[b]*Recovery of ALC in patients treated with CladT3.5.*

[c]*Week number represents time from start of the study.*

ALC, absolute lymphocyte count; CladT3.5, cladribine tablets 3.5 mg/kg, cumulative dose over 2 years; IQR, interquartile range.

Age≤50 and >50 groups were 20 (10, 38) cells/μL and 13 (8, 24) cells/μL, respectively. CD19+ B lymphocyte levels recovered to normal range by Week 36 (**Figure 1B**; **Supplementary Table 1**). Year 2 nadir for CD19+ B lymphocytes occurred at Week 52 (4 weeks following start of Year 2 treatment; median [IQR] for Age ≤50: 31 [20, 58] cells/μL; Age >50: 33 [18, 78] cells/μL; both below LLN), with recovery to normal levels by Week 96.

CD4+ T Lymphocytes

Following treatment with cladribine tablets 3.5 mg/kg, Year 1 nadir for CD4+ T lymphocytes occurred at Week 16; median (IQR) for the Age ≤50 and Age >50 groups were 391 (290, 584) cells/μL and 377 (302, 538) cells/μL, respectively. A small increase was observed in CD4+ T lymphocytes after Week 16, and levels remained in the normal range until end of Year 1. In Year 2, nadir for CD4+ T lymphocytes was below LLN in both age groups: median (IQR) was 281 (206, 410) cells/μL for the Age ≤50 group at Week 60 (12 weeks following start of Year 2 treatment) and 250 (189, 423) cells/μL for the Age >50 group at Week 72 (24 weeks following start of Year 2 treatment); levels gradually increased and reached normal range by Week 96 (**Figure 1C**; **Supplementary Table 2**).

CD8+ T Lymphocytes

Following treatment with cladribine tablets 3.5 mg/kg, Year 1 nadir for CD8+ T lymphocytes occurred at Week 16 (median [IQR]: 260 [151, 383] cells/μL) in the Age ≤50 group and remained in the normal range. In the Age >50 group, Year 1 nadir occurred at Week 9 (median [IQR]: 191 [120, 215] cells/μL), and recovered to the normal range by end of Year 1. Year 2 nadir occurred at Week 72 (24 weeks following start of Year 2; median [IQR]: 233 [160, 336] cells/μL) in the Age ≤50 group. In the Age >50 group Year 2 nadir occurred later at Week 96 (48 weeks following start of Year 2; median [IQR]: 199 [156, 389] cells/μL). CD8+ T lymphocytes remained in the normal range in Year 2 (**Figure 1D**; **Supplementary Table 3**).

Grade ≥3 Lymphopenia

Incidence of Grade ≥3 Lymphopenia Over 96 Weeks

In Year 1, following treatment with cladribine tablets 3.5 mg/kg, Gr≥3L was reported in 8.3% and 10.0% of patients in the Age ≤50 and >50 groups, respectively; in Year 2, this increased to 18.7% and 20.0% (**Table 3**). Grade 4 lymphopenia did not occur with cladribine tablets 3.5 mg/kg in Year 1; in Year 2, two (0.3%) patients in the Age ≤50 group and one (1.0%) patient in the Age >50 group experienced Grade 4 lymphopenia. Through Week 96, the overall incidence of Gr≥3L in patients treated with cladribine tablets 3.5 mg/kg was 25.2% (205/813) in the Age ≤50 group and 27.3% (30/110) in the Age >50 group; the mean (standard deviation [SD]) number of Gr≥3L episodes per patient in the respective age groups were 1.6 (1.1) and 1.9 (1.0). **Figure 2** shows the time to onset of first Gr≥3L in the Age ≤50 and >50 groups.

Time to Recover From Grade ≥3 Lymphopenia

Median (95% CI) time to improvement from Gr≥3L to Grade ≤2 lymphopenia was 5.7 (5.3, 6.1) weeks in the Age ≤50 group and 6.4 (5.3, 8.9) weeks in the Age >50 group. Among patients with Gr≥3L, 87.3% (179/205) in the Age ≤50 group and 80% (24/30) in the Age >50 group remained in the study for 96 weeks. However, whether lymphopenia was the reason for discontinuation in 12.7–20% of patients could not be established given the challenge of

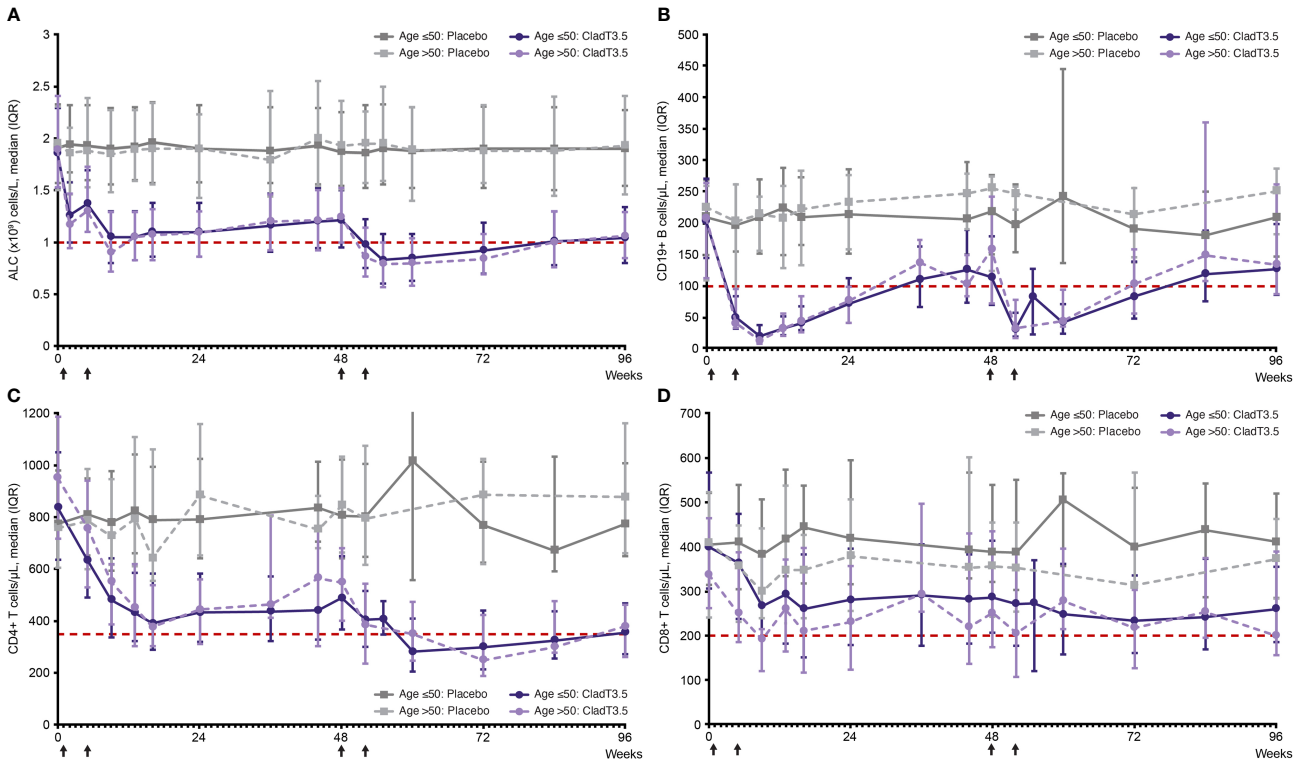


FIGURE 1 | Absolute lymphocyte count and lymphocyte subset levels over time (Week 0–96) from first dose of cladribine tablets 3.5 mg/kg or placebo by age, ≤50 years and >50 years. **(A)** absolute lymphocyte count, **(B)** CD19+ B, **(C)** CD4+ T, and **(D)** CD8+ T lymphocyte subsets. *Notes*: Treatment weeks are indicated by black arrows. Only visits with a sample size ≥10 are shown (On the lymphocyte subset graphs no data plotted for Age ≤50: Placebo [Weeks 36 and 55]; Age >50: Placebo [Weeks 36, 55, 60 and 84]; Age >50: CladT3.5 [Week 55]). Reference line (red) in **(A)** corresponds to ALC lower limit of normal of 1.02 x 10^9/L. Reference line (red) in **(B–D)** corresponds to lower limit of normal of 100, 350 and of 200 cells/µL, for CD19+ B, CD4+ T and CD8+ T lymphocytes, respectively. ALC, absolute lymphocyte count; CladT3.5, cladribine tablets 3.5 mg/kg; IQR, interquartile range.

retrospectively applying a consistent definition for study discontinuation across different clinical trials.

Viral and Bacterial Infections in Patients With Grade ≥3 Lymphopenia

The overall incidence of viral and bacterial infections in patients with Gr≥3L following cladribine tablets 3.5 mg/kg treatment was 19.71 (16.28–23.87) and 23.78 (14.78–38.25) Adj-TEAE/100PY (95% CI) for the Age ≤50 and Age >50 groups, respectively (**Table 4**). The most common viral and bacterial infections with cladribine tablets 3.5 mg/kg treatment were (Age ≤50 *vs.* Age >50 in Adj-TEAE/100PY [95% CI]): viral upper respiratory tract infection (RTI; 6.43 [4.84–8.53] *vs.* 4.73 [1.97–11.36]), influenza (3.72 [2.62–5.29] *vs.* 4.83 [2.01–11.60]), upper RTI (3.36 [2.32–4.86] *vs.* 4.60 [1.92–11.06]), and herpes zoster (0.76 [0.36–1.59] *vs.* 3.37 [1.27–8.99]; **Table 4**). In both age groups viral and bacterial infections in patients who received cladribine tablets 3.5 mg/kg were mild to moderate in severity with the exception of one patient in the >50 age group who experienced severe pneumonia and bronchitis (**Supplementary Table 4**).

TABLE 3 | Incidence of Grade ≥3 lymphopenia in years 1 and 2 by age group.

Patients, n (%)	Age ≤50 years		Age >50 years	
	Placebo (N=566)	CladT3.5 (N=813)	Placebo (N=75)	CladT3.5 (N=110)
Year 1, n	566	808*	75	110
Grade ≥3, n (%)	2 (0.4)	67 (8.3)	0	11 (10.0)
Year 2, n	516	743	68	105
Grade ≥3, n (%)	1 (0.2)	139 (18.7)	0	21 (20.0)

**5 patients did not have post baseline values.*
CladT3.5, cladribine tablets 3.5 mg/kg, cumulative dose over 2 years.

DISCUSSION

The immune system undergoes significant remodeling during aging due to immunosenescence. Studies characterizing the impact and efficacy of DMTs in older patients with MS are limited. Results from this *post hoc* exploratory analysis demonstrated that cladribine tablets 3.5 mg/kg had a similar effect on ALC and lymphocyte subsets in both older and younger patients over 2 years of treatment. In both groups, ALC and levels of lymphocyte subsets decreased in the weeks following cladribine tablets 3.5 mg/kg dosing in both treatment years, and then gradually increased back to normal levels. Additionally, the

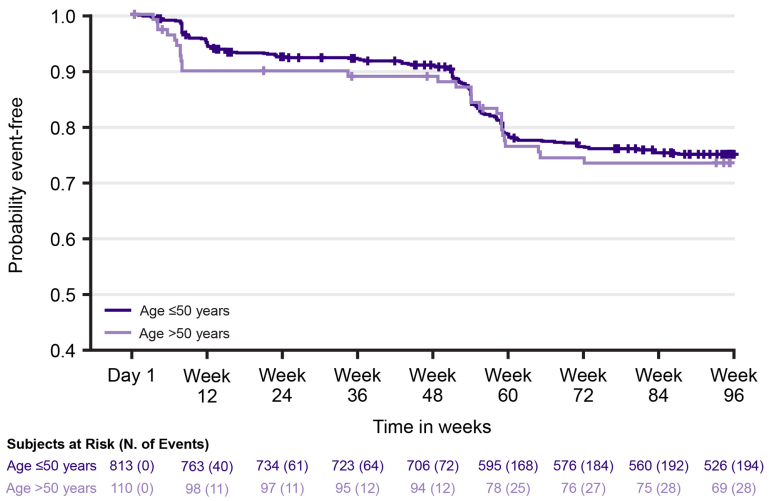


FIGURE 2 | Time to first episode of Grade ≥3 lymphopenia with cladribine tablets 3.5 mg/kg by age group.

TABLE 4 | TEAEs of viral and bacterial infections in years 1 and 2 among patients who reported Grade ≥3 lymphopenia.

Patients, n (%)	Age ≤50 years				Age >50 years			
	Placebo (N=9)	Adj-TEAE/100PY (95% CI)	CladT3.5 (N=205)	Adj-TEAE/100PY (95% CI)	Placebo (N=1)	Adj-TEAE/100PY (95% CI)	CladT3.5 (N=30)	Adj-TEAE/100PY (95% CI)
Any viral or bacterial infections	4 (44.4)	9.67 (3.63–25.76)	105 (51.2)	19.71 (16.28–23.87)	0	0 (0–46.95)	17 (56.7)	23.78 (14.78–38.25)
Viral upper RTI	0	0 (0–6.16)	48 (23.4)	6.43 (4.84–8.53)	0	0 (0–46.95)	5 (16.7)	4.73 (1.97–11.36)
Influenza	1 (11.1)	1.93 (0.27–13.67)	31 (15.1)	3.72 (2.62–5.29)	0	0 (0–46.95)	5 (16.7)	4.83 (2.01–11.60)
Upper RTI	2 (22.2)	3.92 (0.98–15.68)	28 (13.7)	3.36 (2.32–4.86)	0	0 (0–46.95)	5 (16.7)	4.60 (1.92–11.06)
Herpes zoster	0	0 (0–6.16)	7 (3.4)	0.76 (0.36–1.59)	0	0 (0–46.95)	4 (13.3)	3.37 (1.27–8.99)

Only TEAEs with >3 Adj-TEAE/100PY are shown.
Adj-TEAE/100PY, Adjusted-TEAE/100 patient-years; CI, confidence interval; CladT3.5, cladribine tablets 3.5 mg/kg, cumulative dose over 2 years; RTI, respiratory tract infection; TEAEs, treatment-emergent adverse events.

incidence and duration of Gr≥3L with cladribine tablets 3.5 mg/kg was similar in both younger and older patients with MS.

Decreases in ALC and lymphocyte subsets have been observed in MS patients treated with DMTs such as interferons, dimethyl fumarate (DMF), and alemtuzumab (22). In an integrated analysis of 2470 patients with MS treated with DMF, Gr≥3L lasting ≥6 months was observed in 2.2% of patients (23). Marked reductions of lymphocyte subsets have been observed following infusion of alemtuzumab, an anti-CD52 monoclonal antibody, with a near-complete depletion of ALC, CD19+ B, CD4+ T, and CD8+ T lymphocytes observed (24, 25). Recovery to normal range after alemtuzumab infusion may take from 8 months (B lymphocytes) to nearly 3 years (T lymphocytes) (26). Lymphopenia is an anticipated effect of cladribine tablets due to its mechanism of action; however, recovery of ALC and lymphocyte subsets (CD19+ B and CD4+ T) following reduction due to cladribine tablets starts soon after nadir, reaching normal levels within 36–48 weeks of the start of Year 2 treatment; CD8+ T lymphocytes did not fall below LLN (4). The results from this *post hoc* analysis showed similar trends in both ALC and levels of lymphocyte subsets with recovery to normal ranges occurring by the end of the treatment year in both age groups.

A potential concern associated with lymphocyte depletion for some DMTs, especially in older patients, is an increased risk of opportunistic infections such as PML caused by the John Cunningham virus (JCV). In a multinational cohort of patients with MS, the seroprevalence of JCV increased from 49.5% in patients <30 years to 66.5% in patients over 60 years (27). It has been reported that patients with MS over 50 years of age are at greater risk for developing PML following fingolimod and DMF treatment (18). Older age at the start of natalizumab treatment may lead to a shorter time to onset of PML (28). The additive effects of immunosenescence, as well as natalizumab-induced narrowing of the T cell receptor repertoire and reduction of lymphocyte subsets, have been attributed to this shorter time to

PML onset with increasing age (18). No cases of PML have been reported with cladribine tablets to the present date (3). In a prior *post hoc* analysis of patients treated with cladribine tablets 3.5 mg/kg in the monotherapy oral cohort (median age ~36 years), an increased frequency of infections was observed during periods of Gr≥3L; the type of infection events in patients with Gr≥3L did not differ from those occurring outside these episodes (4). This *post hoc* analysis explored the incidence and nature of TEAEs of viral and bacterial infections by age among patients treated with cladribine tablets 3.5 mg/kg who reported Gr≥3L. In this analysis, approximately a quarter of patients treated with cladribine tablets 3.5 mg/kg from each age group experienced at least one episode of transient Gr≥3L. Among patients with Gr≥3L, the rate of certain infection-related TEAEs was numerically higher in older versus younger patients: influenza (4.83 *vs.* 3.72 Adj-TEAE/100PY), upper RTI (4.60 *vs.* 3.36), and herpes zoster (3.37 *vs.* 0.76). The noticeably higher rate of herpes zoster reactivation observed in the Age >50 group is consistent with previous studies, in which viral reactivations were observed in older patients (12, 20).

The current analysis had some limitations. First, it is a *post hoc* analysis of data from previous Phase 3 trials that were not powered to evaluate differences between the younger and older patient groups. Second, most patients (~80%) in this study were treatment naïve prior to study enrollment, which is an unlikely scenario in older patients in the real world. As older patients with MS in the real world are likely to have received prior DMTs, it is unclear how immunosenescence and prior DMT use might impact the effect of cladribine tablets in these patients; this is a subject that requires further research. Recently published real-world studies of cladribine tablets patients (mostly non-elderly) who switched from another DMT showed that cladribine's effect on lymphocyte changes and clinical and magnetic resonance imaging outcomes were broadly similar in those who were previously untreated compared with those previously treated with DMF, fingolimod, beta-interferons, glatiramer acetate, or teriflunomide (29, 30). Adverse events were also shown to be similar in a small group of patients treated with cladribine, ocrelizumab or rituximab after immediate prior natalizumab use. The effect of cladribine tablets after natalizumab is the subject of ongoing Phase 4 CLADRINA trial (NCT04178005). Third, the Age >50 group was notably smaller than the Age ≤50 group (185 *vs.* 1379); as Adj-TEAE/100PY is based on exposure years, a low sample size will lead to reduced exposure time, which will impact TEAE adjustment. Additionally, TEAE data were not adjusted for duration of a Gr≥3L episode and should be interpreted with caution. Fourth, our analysis of older patients was limited by the fact that the CLARITY study included patients only up to 65 years of age at baseline. In the general population more notable declines in immune response have been reported in people over 65 years of age and these have been associated with a higher vulnerability to infections (31, 32). Fifth, it is important to note that the term 'immunosenescence' refers to a quantitative and qualitative decline in immune function with age (33). With aging, there is a marked decline in the production of lymphocytes in the thymus and bone marrow (34). Additionally, qualitative impairments in lymphocytes such as a restricted receptor diversity in T and B cells, and reduced humoral responses against new antigens also occur with aging; these changes contribute to weakened immune response with age (33, 34). Lymphocytes from younger versus older individuals also show distinct gene expression signatures, such as an altered expression of chemokine and cytokine receptors (35). While this current analysis focused on the quantitative differences in lymphocytes among patients treated with cladribine tablets or placebo, qualitative differences in these lymphocytes were not measured. Finally, circulating lymphocytes constitute only ~2% of the total lymphocyte population and, therefore, may not accurately reflect changes that occur within the CNS (22).

CONCLUSION

The findings of this *post hoc* analysis of data from Phase 3 studies of cladribine tablets 3.5 mg/kg (CLARITY, CLARITY Extension and ORACLE-MS) demonstrate that cladribine tablets 3.5 mg/kg had a similar effect on ALC, and lymphocyte subsets (CD19+ B, CD4+ T and CD8+ T) in younger and older patients during 2 years of treatment; steady recoveries following nadir were noted in both age groups. The incidence of transient Gr≥3L following treatment with cladribine tablets 3.5 mg/kg was similar between age groups, with ~25% of patients in either group experiencing at least one episode of Gr≥3L. Among patients treated with cladribine tablets 3.5 mg/kg who experienced at least one episode of Gr≥3L, the rate of certain infection-related TEAEs was numerically higher in the older versus younger patient group.

DATA AVAILABILITY STATEMENT

Any requests for data by qualified scientific and medical researchers for legitimate research purposes will be subject to Merck's Data Sharing Policy. All requests should be submitted in writing to Merck's data sharing portal https://www.merckgroup.com/en/research/our-approach-to-research-and-development/healthcare/clinical-trials/commitment-responsible-data-sharing.html. When Merck has a co-research, co-development, or co-marketing or co-promotion agreement, or when the product has been out-licensed, the responsibility for disclosure might be dependent on the agreement between parties. Under these circumstances, Merck will endeavor to gain agreement to share data in response to requests.

ETHICS STATEMENT

CLARITY (NCT00213135), CLARITY Extension (NCT00641537), and ORACLE-MS (NCT00725985) were conducted in accordance

with the ethical principles of Declaration of Helsinki and the International Conference on Harmonization (ICH) Harmonized Tripartite Guidelines for Good Clinical Practice (GCP). The study protocols were approved by the institutional review boards and relevant ethics committees of participating centers. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conception, design, and methodology: JA, AN, AG, and CL. Acquisition of data: GG, PKC, PV, BW, and TPL. Analysis of data: JA, AN, AG, and CL. Interpretation of data: GG, PKC, PV, BW, JA, AN, AG, CL, and TPL. Writing, review, and/or revision of the manuscript: GG, PKC, PV, BW, JA, AN, AG, CL, and TPL. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.763433/full#supplementary-material

Supplementary Table 1 | Nadir and recovery of CD19+ B lymphocytes in Years 1 and 2 by age group.

Supplementary Table 2 | Nadir and recovery of CD4+ T lymphocytes in Years 1 and 2 by age group.

Supplementary Table 3 | Nadir and recovery of CD8+ T lymphocytes in Years 1 and 2 by age group.

Supplementary Table 4 | Viral and bacterial infections among patients experiencing Gr≥3L, by severity.

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Disease-Modifying Drug Uptake and Health Service Use in the Ageing MS Population

Huah Shin Ng[1†], Jonas Graf[1,2†], Feng Zhu[1], Elaine Kingwell[1,3], Orhan Aktas[2], Philipp Albrecht[2], Hans-Peter Hartung[2,4,5,6], Sven G. Meuth[2], Charity Evans[7], John D. Fisk[8], Ruth Ann Marrie[9,10], Yinshan Zhao[1] and Helen Tremlett[1]*

[1] Department of Medicine, Division of Neurology and the Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, BC, Canada, [2] Department of Neurology, Medical Faculty, University Hospital, Heinrich-Heine-University, Düsseldorf, Germany, [3] Research Department of Primary Care & Population Health, University College London, London, United Kingdom, [4] Brain and Mind Centre, University of Sydney, Sydney, NSW, Australia, [5] Department of Neurology, Medical University of Vienna, Vienna, Austria, [6] Department of Neurology, Palacky University in Olomouc, Olomouc, Czechia, [7] College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada, [8] Nova Scotia Health Authority and the Departments of Psychiatry, Psychology and Neuroscience, and Medicine, Dalhousie University, Halifax, NS, Canada, [9] Department of Internal Medicine, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada, [10] Department of Community Health Sciences, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada

Background: Evidence regarding the efficacy or effectiveness of the disease-modifying drugs (DMDs) in the older multiple sclerosis (MS) population is scarce. This has contributed to a lack of evidence-based treatment recommendations for the ageing MS population in practice guidelines. We examined the relationship between age (<55 and ≥55 years), DMD exposure and health service use in the MS population.

Methods: We conducted a population-based observational study using linked administrative health data from British Columbia, Canada. We selected all persons with MS and followed from the most recent of their first MS or demyelinating event, 18th birthday or 01-January-1996 (index date) until the earliest of emigration, death or 31-December-2017 (study end). We assessed DMD exposure status over time, initially as any versus no DMD, then by generation (first or second) and finally by each individual DMD. Age-specific analyses were conducted with all-cause hospitalizations and number of physician visits assessed using proportional means model and negative binomial regression with generalized estimating equations.

Results: We included 19,360 persons with MS (72% were women); 10,741/19,360 (56%) had ever reached their 55th birthday. Person-years of follow-up whilst aged <55 was 132,283, and 93,594 whilst aged ≥55. Any DMD, versus no DMD in the <55-year-olds was associated with a 23% lower hazard of hospitalization (adjusted hazard ratio, aHR0.77; 95%CI 0.72-0.82), but not in the ≥55-year-olds (aHR0.95; 95%CI 0.87-1.04). Similar patterns were observed for the first and second generation DMDs. Exposure to any (versus no) DMD was not associated with rates of physician visits in either age group (<55 years: adjusted rate ratio, aRR1.02; 95%CI 1.00-1.04 and ≥55 years: aRR1.00; 95% CI 0.96-1.03), but variation in aRR was observed across the individual DMDs.

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Angel Perez Sempere, Hospital General Universitario de Alicante, Spain

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Seema Kalra, University Hospitals of North Midlands NHS Trust, United Kingdom
Emilio Portaccio, Careggi University Hospital, Italy

***Correspondence:**
Helen Tremlett
helen.tremlett@ubc.ca

†These authors share first authorship

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Conclusion: Our study showed beneficial effects of the DMDs used to treat MS on hospitalizations for those aged <55 at the time of exposure. In contrast, for individuals ≥55 years of age exposed to a DMD, the hazard of hospitalization was not significantly lowered. Our study contributes to the broader understanding of the potential benefits and risks of DMD use in the ageing MS population.

Keywords: ageing, cohort studies, disease-modifying drugs, health services, hospitalization, multiple sclerosis, physician services

INTRODUCTION

Multiple sclerosis (MS) is a chronic, immune-mediated disease characterized by demyelination and neurodegeneration affecting both brain and spinal cord. While most people will be diagnosed with MS between the ages of 20-50 years, the average age range of people living with MS in North America is between 55 and 65 years (1, 2). Despite this, people with MS aged 55 years or older have often been excluded from clinical trials testing the efficacy of disease-modifying drugs (DMDs) (1–4). While short-term MS clinical trials showed limited benefits of taking DMD after age 53 years (5), and the potential for harm (e.g., higher neoplasm risk, especially after age 45 years) (6), all based on meta-analyses, the evidence regarding the long-term efficacy or effectiveness and safety profile of DMDs in the older MS population is scarce (7, 8). This has contributed to a lack of treatment recommendations for the ageing MS population in practice guidelines (3, 4).

Health administrative data which captures health care use information offers opportunity to assess the real-world effectiveness of the DMDs used to treat MS. This approach has been successfully applied to examine the safety and effectiveness of the DMDs in the general, or healthcare insured, MS population (9–13).

In this study, we accessed population-based health administrative data captured over a 22-year period in the province of British Columbia, Canada, to examine the relationship between age (<55 and ≥55 years), DMD exposure and health service use in the MS population. We examined age as a dynamic process for each person, by dividing the individual's time spent before and after reaching age 55 years.

MATERIALS AND METHODS

Data Sources

We conducted a population-based observational study using linked administrative health data. These prospectively collected data covered the population of British Columbia, comprising 4.64 million residents, and representing around 13% of the Canadian population (14). The linked data comprised five datasets: (i) the provincial health insurance registry (15) providing demographic information for each individual, including sex, date of birth, residency status and location (three digit postal codes); (ii) vital statistics data (16) providing the date of death; (iii and iv) physician billing (17) and discharge abstract databases (18) capturing all physician visits and hospitalizations, with reasons for the visit or admission coded using the International Classification of Diseases (ICD)-9/10 system; and (v) the provincial prescription database (PharmaNet) (19) capturing all prescription drugs dispensed at outpatient and community pharmacies.

Study Population

We selected all persons with MS by using a validated algorithm with the cases defined as having at least 3 MS diagnostic codes (ICD-9/10 340/G35) in the hospital and/or physician data or an MS DMD record in the prescription data, as outlined previously (20–22). We assigned an index date to each person based on the most recent date of: the first MS or demyelinating event recorded in the hospital, physician or prescription data (**Supplementary Tables 1**, **2**), or 01-January-1996 (the date when the provincial prescription data first became available), or the person's 18th birthday. All persons required at least one year of residency in British Columbia before the index date, and were followed from the index date until the study end date defined as the earliest of emigration from the province, death or 31-December-2017.

We determined the cohort characteristics at the index date, including age, calendar year, sex, and socioeconomic status (reported as neighborhood-level income quintiles according to a person's three-digit postal codes by linkage to census data) (23). The burden of comorbidity was measured using a modified Charlson Comorbidity Index based on the diagnoses captured in the hospital and physician data during the one-year before the index date, with hemiplegia and paraplegia excluded to avoid misclassifying symptoms related to MS as comorbidity (24, 25).

DMD Exposure

The DMDs available during our study period (**Supplementary Table 2**) included the first generation DMDs – beta-interferon and glatiramer acetate, and the second generation DMDs – natalizumab, fingolimod, dimethyl fumarate, teriflunomide, alemtuzumab, daclizumab, and ocrelizumab. We grouped all beta-interferon products together as one class. We assessed DMD exposure status as a time-varying variable, initially as any versus no DMD, then by generation (first or second), and finally by individual DMDs. Neither daclizumab nor ocrelizumab were included in the assessment of individual DMDs due to the small number of individuals (<6) exposed over the study period, preventing the derivation of reliable estimates.

We determined the DMD exposure periods according to the number of days supplied for each individual DMD. A DMD was considered as being discontinued when there were no further

dispensations for the DMD for greater than 90 days. The discontinuation date was defined as the last DMD prescription fill date plus the number of days supplied, with a 30-day grace period applied (26). The exception was for alemtuzumab and ocrelizumab, whereby periods of exposure were defined as one year (for alemtuzumab) and six months (for ocrelizumab), from the date of first supply, plus a 30-day grace period if no further DMD fills occurred. Finally, as persons could not be on more than one DMD at the same time, once a person filled a new DMD prescription, then the previous DMD was considered discontinued. DMD exposure was assessed as a time-varying variable (i.e. a person's DMD exposure status was allowed to change over time).

Age Grouping

We assessed each person's current age as a time-varying variable, grouped as <55 or ≥55 years old. This age grouping was selected partly because older individuals (≥55 years) have often been excluded from enrolling in MS clinical trials (1–4), and partly because the prevalence of persons with MS aged 55 and above has risen in recent years (20, 27), such that these individuals represent a growing yet understudied group.

Outcomes

The outcome measures were all-cause hospitalizations and number of physician visits.

Hospitalization data included day surgery/minor procedures (but not drug infusions e.g., for natalizumab or alemtuzumab) (28). For the hospitalizations, any overlapping admissions or any new admission that occurred within one day of the previous hospitalization were counted as a single event (10, 29). For the physician visits, multiple claims with the same primary ICD code captured on the same day were counted as a single visit (10, 29). Neurologist visits were also excluded from the count as the number of these visits was anticipated to be higher in persons exposed to a DMD (versus no DMD) as part of routine care (4) (other physician specialties cannot prescribe an MS DMD in British Columbia). In addition, any pregnancy-related encounters (hospitalizations or physician visits based on the primary ICD code) were not included as an outcome as DMD cessation was expected to be common during pregnancy (4).

Statistical Analyses

We described the cohort characteristics at the index date by age group (<55 versus ≥55 years old at the index date) and DMD exposure (at any time during follow-up), using counts and percentages for the categorical variables, and means and standard deviations for the continuous variables. Person-years of follow-up by current age group at the time of exposure for each individual DMD was also reported.

We included an interaction term between DMD exposure and current age group (<55 or ≥55 years) at the time of follow-up to estimate the age-specific associations between DMD exposure and outcomes (all-cause hospitalizations and the number of physician visits). For each analysis, the period of 'no DMD exposure' was used as the reference category.

All-cause hospitalizations were assessed using proportional means models with robust sandwich variance estimates (30). Models were adjusted for sex, socioeconomic status (quintiles), age (continuous) and calendar year (continuous) at the index date, and for the Charlson comorbidity score, categorized as: 0, 1, 2, ≥3, and updated annually over time. The period of hospitalization was discounted from the follow-up time as a person could not be at risk of another hospitalization during the existing hospital stay. Findings were expressed as adjusted hazard ratios (aHRs) with the corresponding 95% confidence intervals (CIs).

The number of physician visits were examined using negative binomial regression models fitted by generalized estimating equations with an exchangeable working correlation matrix (31). The number of physician visits were calculated annually, or by DMD exposure periods when there were changes in DMD status within a year. An offset was included in the model to account for the variable length of time periods (log of person-time). Models were adjusted for sex and socioeconomic status (quintiles) at the index date, and the following covariates over time (updated on an annual basis) including age (continuous), Charlson comorbidity score (categorized as: 0, 1, 2, ≥3) and calendar year (continuous; to account for any secular changes in healthcare use). Findings were expressed as adjusted rate ratios (aRRs) with the corresponding 95% CIs.

A complementary analysis was also performed to describe the DMD exposure status for any person who had reached age 55 years at any time before the study end date.

We conducted the statistical analyses using SAS software version 9.4 (SAS Institute, Cary, NC, USA).

Study Registration and Ethical Approval

This study was registered with ClinicalTrials.gov (NCT04472975). We obtained ethics approval from the University of British Columbia's Clinical Research Ethics Board (H18-00407).

RESULTS

Cohort Characteristics

We identified a total of 19,360 persons with MS (72.0% were women). Almost half (44.1%, 8,533/19,360) of the cohort entered the study between 1996-1999, with the remainder entering between 2000-2017. The mean follow-up time was 11.7 years (SD 7.3). At the index date, 78.7% (15,235/19,360) were aged <55 years and 21.3% (4,125/19,360) were ≥55 years (**Table 1**). Of those aged <55 years at the index date, 29.7% (4,526/15,235) filled a DMD prescription during follow-up, whereas 5.0% (206/4,125) of persons aged ≥55 years at the index date did so. Within both age groups, those treated with an MS DMD were approximately 4-5 years younger relative to those who were untreated. For example, for those under age 55 years at the index date, the mean age was 36.1 years [SD 8.7] for those ever DMD exposed during follow-up, versus 40.5 years [SD 8.9] for those unexposed.

TABLE 1 | Characteristics of the multiple sclerosis study population by age group at the index date (<55 versus ≥55 years old) and by exposure to a disease-modifying drug at any time during follow-up, n=19,360.

Characteristics	Age at Index Date <55 Years, n=15,235		Age at Index Date ≥ 55 Years, n=4,125	
	DMD-Treated[a] n=4,526	Not Treated[a] n=10,709	DMD-Treated[a] n=206	Not Treated[a] n=3,919
Sex, n (%)				
Women	3,324 (73.4)	7,868 (73.5)	145 (70.4)	2,603 (66.4)
Men	1,202 (26.6)	2,841 (26.5)	61 (29.6)	1,316 (33.6)
Age at index date in years, mean (SD)	36.1 (8.7)	40.5 (8.9)	58.9 (4.6)	64.5 (8.0)
Socioeconomic status[b], n (%)				
1 (lowest income quintile)	876 (19.4)	2,037 (19.0)	38 (18.4)	812 (20.7)
2	839 (18.5)	2,075 (19.4)	31 (15.0)	750 (19.1)
3	953 (21.1)	2,149 (20.1)	39 (18.9)	790 (20.2)
4	958 (21.2)	2,311 (21.6)	48 (23.3)	777 (19.8)
5 (highest income quintile)	888 (19.6)	2,083 (19.5)	50 (24.3)	758 (19.3)
Unavailable	12 (0.3)	54 (0.5)	<6	32 (0.8)
Comorbidity score[c], n (%)				
0	3,820 (84.4)	8,552 (79.9)	154 (74.8)	2,525 (64.4)
1	553 (12.2)	1,585 (14.8)	35 (17.0)	806 (20.6)
2	121 (2.7)	388 (3.6)	11 (5.3)	335 (8.5)
≥ 3	32 (0.7)	184 (1.7)	6 (2.9)	253 (6.5)
Calendar year at index date, n (%)				
1996-1999	1,479 (32.7)	5,132 (47.9)	50 (24.3)	1,872 (47.8)
2000-2009	1,818 (40.2)	3,374 (31.5)	73 (35.4)	1,152 (29.4)
2010-2017	1,229 (27.2)	2,203 (20.6)	83 (40.3)	895 (22.8)
Follow-up[a] time in years,				
median (Q1, Q3)	12.2 (5.9, 18.6)	12.0 (5.4, 20.0)	8.2 (3.9, 13.3)	8.6 (4.0, 14.7)
mean (SD)	12.2 (7.0)	12.2 (7.5)	9.3 (6.5)	9.7 (6.6)
Number of different DMD prescriptions filled during the follow-up[a]				
1	2,865 (63.3)	N/A	171 (83.0)	N/A
2	1,194 (26.4)		30 (14.6)	
≥ 3	467 (10.3)		<6	
First DMD prescription, n (%)				
Beta-interferon[d]	2,833 (62.6)	N/A	122 (59.2)	N/A
Glatiramer acetate	1,080 (23.9)		48 (23.3)	
Natalizumab	63 (1.4)		<6	
Fingolimod	31 (0.7)		<6	
Dimethyl fumarate	300 (6.6)		13 (6.3)	
Teriflunomide	181 (4.0)		15 (7.3)	
Alemtuzumab	36 (0.8)		<6	
Daclizumab	<6		<6	
Ocrelizumab	<6		<6	
Number of individuals ever exposed, by type of DMD, during follow-up[a], n (%)				
First generation DMDs –any[e]	3,953 (87.3)	N/A	171 (83.0)	N/A
Beta-interferon[d]	3,016 (66.6)		124 (60.2)	
Glatiramer acetate	1,655 (36.6)		64 (31.1)	
Second generation DMDs – any[e]	1,703 (37.6)		53 (25.7)	
Natalizumab	277 (6.1)		9 (4.4)	
Fingolimod	416 (9.2)		<6	
Dimethyl fumarate	736 (16.3)		22 (10.7)	
Teriflunomide	497 (11.0)		23 (11.2)	
Alemtuzumab	178 (3.9)		<6	
Daclizumab	6 (0.1)		<6	
Ocrelizumab	<6		<6	

Key: SD, standard deviation; DMD, disease-modifying drug, N/A, not applicable.

As per data privacy and access agreements, small cell size (<6 individuals within any group) are suppressed.

[a]*Follow-up was from index date until the study end date (up to December 31st 2017).*

[b]*Socioeconomic status is reported by neighborhood income quintiles according to a person's three-digit postal codes (closest available to the index date).*

[c]*Comorbidity was measured using the modified Charlson Comorbidity Index (exclude hemiplegia/paraplegia to avoid misclassifying MS complications as comorbidity) based on the diagnoses captured in the hospital and physician data during the one-year before the index date.*

[d]*All beta-interferon products were grouped together as one class.*

[e]*Some people were exposed to >1 DMD; hence the sum of the individual first or second generation DMDs exceeds the sum of any first or second generation DMD.*

TABLE 2 | Person-years of follow-up in the multiple sclerosis cohort by each person's current age, grouped as <55 or ≥55 years old, and by disease-modifying drug exposure status.

Person-Years of Follow-Up	Person's Current Age	
	<55 Years [1]	**≥55 Years [2]**
During periods of exposure to:		
Any DMDs	20,555.5	4,414.8
Any first generation DMDs	17,180.4	3,842.8
Beta-interferon[a]	12,413.7	2,911.9
Glatiramer acetate	4,766.6	930.8
Any second generation DMDs	3,375.2	572.0
Natalizumab	745.6	85.5
Fingolimod	871.4	115.6
Dimethyl fumarate	1,051.0	195.4
Teriflunomide	489.0	168.5
Alemtuzumab	216.2	6.4
Daclizumab	<6	<6
Ocrelizumab	<6	<6
No DMD	111,727.6	89,179.3
Total person-years of follow-up	132,283.1	93,594.1

Key: DMD, disease-modifying drug.

[a]*All beta-interferon products were grouped together as one class.*

Total cohort size=19,360. Of these, by the study end n=10,741/19,360 (55.5%) had ever reached their 55th birthday, with n=4,125/10,741 (38.4%) doing so by the index date and n=6,616/10,741 (61.6%) during follow-up. The remainder, n=8,619/19,360 (44.5%) never reached their 55th birthday by the study end. Thus, n=6,616 individuals contributed follow-up time to both columns [1] and [2], n=8,619 only to column [1] and n=4,125 only to column [2].

Irrespective of DMD exposure status during follow-up, the comorbidity burden (measured using the modified Charlson Comorbidity Index) was lower for persons <55 years at the index date, relative to those ≥55 years old. With respect to DMD exposure status, the comorbidity burden at the index date was lower in the DMD-treated group compared to the non-treated group. For example, for those age ≥55 years at the index date, 52/206 (25.2%) had a comorbidity in the DMD-treated group, versus 1,394/3,919 (35.6%) in the non-treated group. The socio-economic quintiles were generally evenly distributed across all four groups (**Table 1**).

Of those ever filling a DMD prescription during follow-up, the proportion exposed to a first generation DMD was similar regardless of age at the index date. Whereas, a higher proportion of the younger (<55 years) MS cases were exposed to a second generation DMD (37.6%, 1,703/4,526), compared to older individuals (≥55 years; 25.7%, 53/206). A higher proportion of younger persons with MS (<55 years at the index date) also switched between DMDs at least once during follow-up (36.7%; 1,661/4,526), compared to older individuals (≥55 years; 17.0%, 35/206).

As younger persons (<55 years) could transition to the older age group (≥55 years) throughout our >20-year study period, an overview of the person-years of follow-up by each person's current age group and DMD exposure status is shown in **Table 2**. Not unexpectedly, the number of person-years of follow-up was higher in persons with a current age of <55 (versus ≥55 years), irrespective of DMD exposure status. Of note the person-years of follow-up for certain DMDs were modest particularly in the older (≥55 years) age group.

Hospitalizations

Any DMD, relative to no DMD, was associated with a 23% lower hazard of hospitalization for those aged <55 years at the time of DMD exposure (aHR 0.77; 95%CI 0.72-0.82), but was not for those aged ≥55 years (aHR 0.95; 95%CI 0.87-1.04), **Figure 1**. Similar trends were observed for the first generation DMDs, where a 22% significantly lower hazard was observed for those aged <55 years, and, for the second generation DMDs, a 27% significantly lower hazard. Neither of these findings reached significance for those aged ≥55 years at the time of DMD exposure, where the lower hazard ranged from 3% to 20%. When the DMDs were assessed individually, the hazard of hospitalization for those aged <55 years at the time of exposure ranged from a 9% lower hazard for natalizumab, to 10% for alemtuzumab, 18% for glatiramer acetate, 24% for beta-interferon, 30% for dimethyl fumarate, 34% for teriflunomide, and 37% for fingolimod. All reached statistical significance, except for natalizumab and alemtuzumab, although the 95% confidence intervals were also very wide for these two DMDs. For person's aged ≥55 years at the time of DMD exposure, the corresponding HRs did not reach significance, except for fingolimod, which was associated with a 37% lower hazard of hospitalization. All results for the DMD exposure and hospitalizations analyses by age group are shown in **Figure 1**.

Physician Visits

While exposure to any DMD (versus no DMD) was not associated with altered rates of physician visits in either age group (<55 years: aRR 1.02; 95%CI 1.00-1.04 and ≥55 years: aRR 1.00; 95%CI 0.96-1.03), variation was observed across the individual DMDs (**Figure 2**). A 27-33% higher rate of physician visits was observed during exposure to a second generation DMD, alemtuzumab, reaching significance in the younger, but not older population. In contrast, exposure to another second generation DMD, fingolimod was associated with a significantly lower rate of physician visits, by 12%, for those <55 years. More modest differences were seen for the first

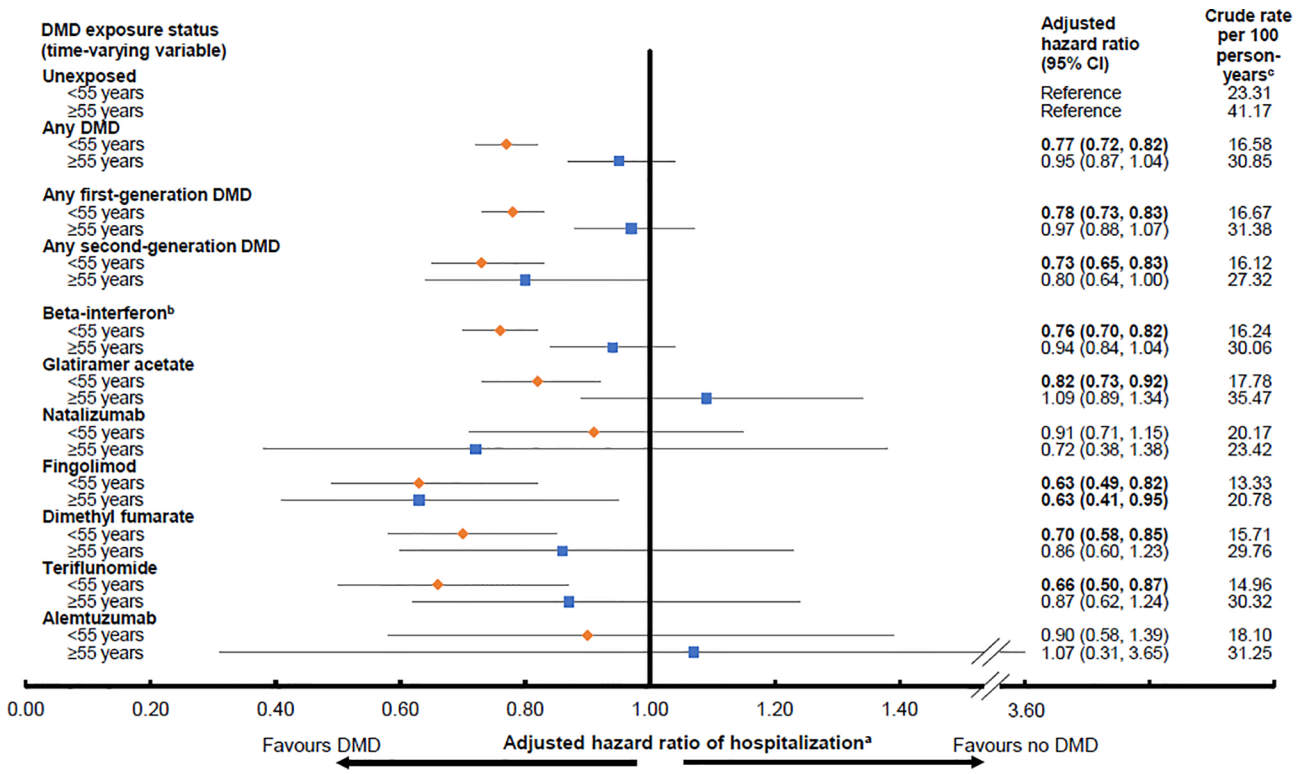

FIGURE 1 | Exposure to a disease-modifying drug for multiple sclerosis and hazard of hospitalization by age group (<55 or ≥55 years old). Key: CI, confidence interval; DMD, disease-modifying drug. Bold indicates p<0.05. [a]Results were adjusted for sex, socioeconomic status (quintiles), age (continuous) and calendar year (continuous) at the index date, and for Charlson comorbidity score (categorical: 0, 1, 2, ≥3) over time (updated on annual basis). Hazard ratios were estimated by introducing interaction terms between current age group (<55 versus ≥55 years at the time of exposure) and the DMD exposure variables shown in the Figure. [b]All beta-interferon products were grouped together as one class. [c]Person-years of follow-up for the calculation of crude rate were as per except that the duration of a hospitalization was discounted from the follow-up time.

generation DMDs, but for those aged <55 years, with beta-interferon associated with a 7% higher rate of physician visits and glatiramer acetate with an 8% lower rate. All results for the DMD exposure and physician visits analyses by age group are shown in **Figure 2**.

Complementary Analysis

By the study end 10,741 persons had ever reached their 55th birthday, with 38.4% (n=4,125/10,741) doing so by the index date and 61.6% (n=6,616/10,741) during follow-up. Approximately 15% (n=1,657/10,741) were exposed to a DMD at any time point during follow-up (**Supplementary Table 3**). While 12% (n=1,302/10,741) had their first DMD before age 55 years, nearly half of these (n=596/1,302) were no longer taking DMD once aged ≥55 years. In total, over 3% (n=355/10,741) of persons initiated their first DMD at the age of 55 years or older.

DISCUSSION

We assessed the effect of current age on the association between DMD exposure and health service utilization in a population-based MS cohort with over 200,000 person-years of follow-up, and all within a universal healthcare setting. Exposure to any DMD or to any first generation DMD (versus no DMD) was associated with a 22-23% lower hazard of hospitalization for those aged <55 years at the time of exposure, while exposure to any second generation DMD was associated with a 27% lower hazard of hospitalization. In contrast, in older adults (≥55 years), DMD exposure, whether assessed as any DMD or any first or second generation or even by individual DMDs (versus no DMD), was generally not associated with a lower risk of hospitalization. Finally, while regardless of age (<55 or ≥55 years), exposure to any DMD, relative to no DMD, was not associated with an altered rate of physician visits, considerable variation was observed across the individual DMDs. Our findings offer insights into the effects of ageing on the relationship between the DMDs used to treat MS and health services in the real-world setting.

While several studies have assessed the relationship between the MS DMDs and healthcare utilization (9–12), we were unable to find another study to compare our age-specific findings. A study from the United States co-authored by a pharmaceutical manufacturer of an MS DMD examined patterns of healthcare

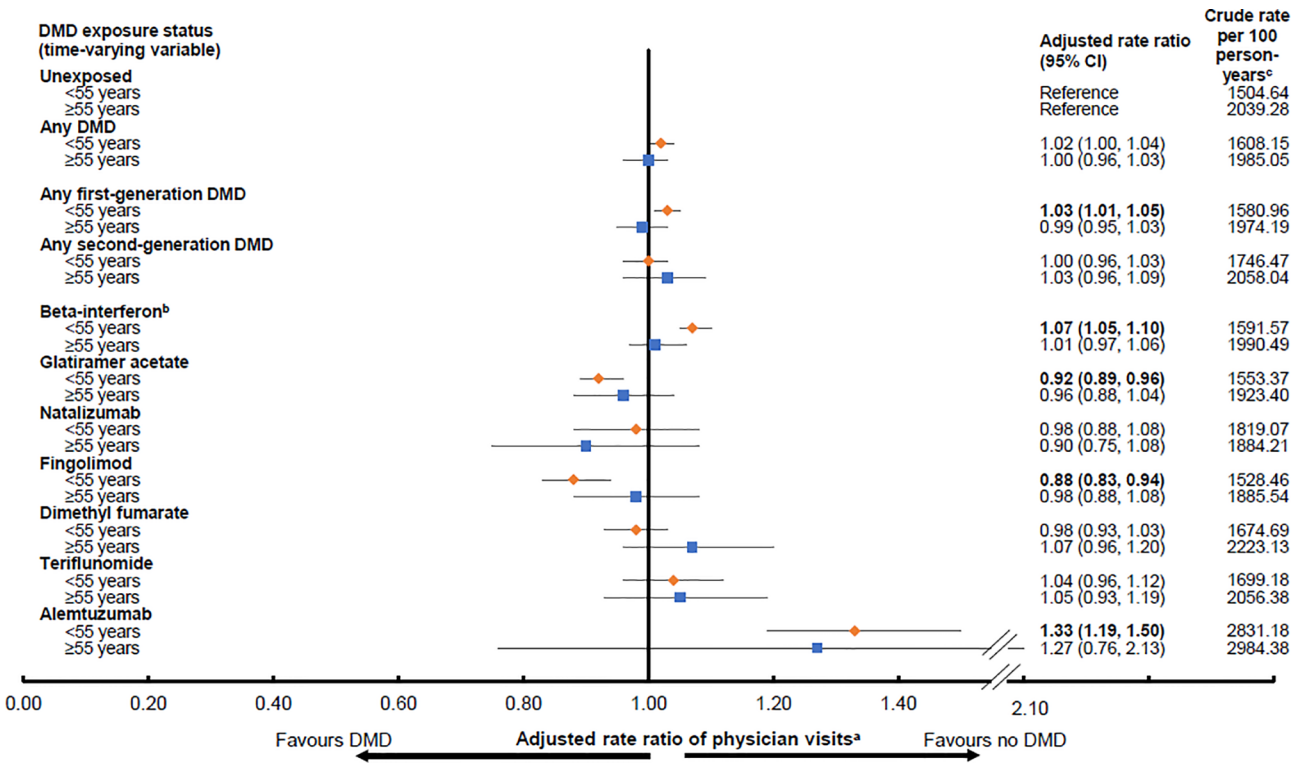

FIGURE 2 | Exposure to a disease-modifying drug for multiple sclerosis and rates of physician visits[d] by age group (<55 or ≥55 years old). Key: CI, confidence interval; DMD, disease-modifying drug. Bold indicates p<0.05. [a]Results were adjusted for sex and socioeconomic status (quintiles) at the index date, and the following characteristics over time on a yearly basis: age (continuous), calendar year (continuous), and Charlson comorbidity score (categorical: 0, 1, 2, ≥3). Rate ratios were estimated by introducing interaction terms between current age group (<55 versus ≥55 years at the time of exposure) and the DMD exposure variables shown in the Figure. [b]All beta-interferon products were grouped together as one class. [c]Person-years of follow-up are shown in **Table 2** and were used to calculate the crude rates. [d]As outlined in the study methods, neurologist visits were excluded, as were pregnancy-related visits.

utilization in 28,427 persons with MS by insurance type (commercial versus the federal insurance plan, Medicare Advantage) within different age groups (32). However, the study was cross-sectional in design, spanning just one-year and was restricted to persons under 65 years of age. The authors found that MS persons with a federal insurance plan had a significantly higher mean count of all-cause inpatient and ambulatory visits compared to those with a commercial insurance plan. While findings were consistent across age groups, at least once age 30 years was reached, the authors did not report which individual DMDs contributed to these findings (32). Efforts have also been made to examine the effects of ageing on the efficacy of DMDs by using data from clinical trials. However, there are some limitations and challenges with these studies. One example is a meta-analysis which included 26 clinical trials of 14 different DMDs published between 1995 and 2019, and comprised 28,082 relapsing-remitting MS persons. Authors found no statistically significant associations between age and reduction(s) in disease activity (measured as annualized relapse rates and magnetic resonance imaging metrics, such as gadolinium-enhancing lesions and T2 lesions) when the DMD- and placebo-treated groups were compared (33). However, as individual-level data were inaccessible, this meta-analysis had to rely on group-level average ages as reported within each clinical trial to examine potential age-related differences in DMD efficacy (33). Moreover, the original clinical trials were not designed to examine DMD efficacy in the ageing population and individuals older than 55 years were excluded from enrollment, such that the average ages of participants ranged from 33 to 40 years (33).

The differences we found in the association between DMD exposure and the hazard of hospitalizations by age group (<55 versus ≥55 years old) does concur with broader observations from both natural history studies of MS and the MS clinical trials. For example, clinical trials have demonstrated beneficial effects of DMDs on reducing or preventing MS relapses in the short-term (34), which may in turn lower the risk of hospitalization (9, 35–38). However, the frequency of MS relapses naturally decreases over time and with age (39, 40), being less common in older individuals, particularly after 60 years of age (2). Furthermore, the DMDs appear less efficacious and less effective in progressive MS (primary or secondary) (41–43) and a higher proportion of the older MS population will have progressive MS (1, 2, 39). The longer-term effects of the DMDs in preventing disability associated with disease progression and ageing is uncertain (2). For example, a study conducted in British Columbia, Canada, showed that exposure to beta-

interferon (versus no exposure) was not associated with a lower hazard of reaching an Expanded Disability Status Scale [EDSS] score of 6 in older MS adults, aged≥50 years (44). Further, a meta-analysis, which included 38 clinical trials of 13 different DMDs with over 28,000 MS persons, showed that the effects of DMDs on MS disability progression was strongly dependent on age, with limited benefits of receiving DMDs after age 53 years (5).

In our study, we found that exposure to any DMD was not associated with differences in the rate of physician visits by age group (<55 versus ≥55 years old), although variation across individual DMDs was observed, especially in the younger age group (<55 years). Use of the MS DMDs typically requires regular laboratory testing and safety-related monitoring. Therefore, it is possible that any potential benefits on, or decreases in, physician visits or hospitalizations related to an anticipated benefit of the DMDs on disease activity may be offset by the increase in safety-related monitoring (10). Higher rates of physician visits, ranging from 27-33%, were observed in both age groups (<55 and ≥55 years old at the time of exposure) while exposed to alemtuzumab, although this failed to reach significance in the older age group. Alemtuzumab requires regular monthly monitoring due to the risk of adverse events, such as autoimmune disorders, which may contribute to these higher rates (45). Similarly, exposure to beta-interferon, which requires regular monitoring for liver and thyroid function (46), was associated with a higher rate of physician visits. However, this was a much more modest 7% and only demonstrated in the younger age group (<55 years old at the time of exposure). In contrast, exposure to glatiramer acetate, which requires no formal laboratory testing (47), was associated with an 8% lower rate of physician visits, and exposure to fingolimod was associated with a 12% lower rate (again in the <55 year old age group only), despite the necessity of regular biochemical liver testing (48). Fingolimod is generally reserved as a second-line therapy in Canada, and a lower rate of physician visits may be due to decreases in disease activity.

Complementary Descriptive Analysis - DMD Use in the Older Age Group

Currently, it remains unclear if DMD treatment should be continued (or even started) in the MS population aged 55 years or older. To address this, a large phase 4 randomized controlled DMD treatment discontinuation trial is currently underway in the Unites States which includes persons ≥55 years old, and its findings may add crucial knowledge to this emerging aspect of MS care (49). While, as expected, a smaller proportion of our older (versus younger) persons with MS were DMD exposed (22, 32, 50), we also observed that the proportions of persons continuing or discontinuing DMD were similar. Specifically, the proportions of persons who initiated their first DMD before age 55 years, and who continued or discontinued once aged ≥55 years were rather similar (n=706/1,302 versus n=596/1,302, respectively) (**Supplementary Table 3**). These findings may reflect individual differences in diseases severity and/or the lack of clear treatment guidelines for the older patient population in clinical practice.

Strengths and Limitations

Our study has both strengths and limitations. Given that some of the newer second-generation DMDs only became widely available towards the end of our study, the total person-years exposed was modest for certain DMDs, particularly in the older (≥55 years) age group. Therefore, we could not examine the specific causes of hospitalizations due to modest event rates by age group which would have hindered derivation of reliable estimates. It is plausible that older individuals are at higher risk of being hospitalized for non-MS than MS-related causes (51). We cannot exclude that clinical or other characteristics that we did not have access to in our administrative data, such as MS disease course, disability level, or cognitive status, or lifestyle factors, such as smoking and alcohol consumption and/or ancestry/ethnicity may have influenced our findings. It remains possible that other confounders not available to us could also be of relevance. However, we were able to adjust for sex, socioeconomic status, and comorbidity burden over time. In addition, we were able to account for the changing treatment status of persons over time by using a longitudinal approach when examining DMD exposure. We consider the potential for selection bias in our study to be minimal given the universal healthcare setting, and our access to comprehensive health care data for all residents of the province, irrespective of ability to pay. Furthermore, we used a validated case definition in our study to select persons with MS. Other strengths of our study are the use of objectively collected population-based data, including linked health administrative information, and the long duration of follow-up (mean 11.7 years).

CONCLUSIONS

Our findings suggest that use of the DMDs to treat MS may be more effective in preventing hospitalizations in younger persons (aged <55 years), compared to older individuals (aged ≥55 years). For those aged <55 years, similar trends were observed for both the first and second generation DMDs. In contrast, a more varied and complex picture evolved when the relationship between DMD exposure and physician visits was examined in the older and younger age groups. This might, in part, reflect the different safety-related monitoring strategies required for the different DMDs. Further studies are warranted in order to expand treatment guidelines for an ageing MS population.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: As we are not the data custodians, we are not authorized to make the data available. With the appropriate approvals, the data may be accessed through the Population Data British Columbia. Requests to access these datasets should be directed to Population Data British Columbia.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of British Columbia's Clinical Research Ethics Board (H18-00407). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HN, JG, and HT interpreted the results and drafted the manuscript. HN, JG, FZ, EK, CE, JF, RM, YZ, and HT conceptualized and designed the study. FZ, EK, CE, JF, RM, YZ, and HT facilitated obtaining funding (PI: Tremlett, CIHR Project and Foundation award). HN, JG, and FZ performed data analysis. All authors revised the manuscript critically for intellectual content, approved the final version to be published, and agreed to be accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.794075/full#supplementary-material

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Evolution of Disease Modifying Therapy Benefits and Risks: An Argument for De-escalation as a Treatment Paradigm for Patients With Multiple Sclerosis

*Brandi L. Vollmer†, Andrew B. Wolf†, Stefan Sillau, John R. Corboy and Enrique Alvarez**

Department of Neurology and Rocky Mountain MS Center, University of Colorado School of Medicine, Aurora, CO, United States

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Edited by:
Aurora Zanghì,
Sant'Elia Hospital, Italy

Reviewed by:
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Paolo Preziosa,
Vita-Salute San Raffaele University, Italy

****Correspondence:***
Enrique Alvarez
enrique.alvarez@cuanschutz.edu

† These authors have contributed equally to this work and share first authorship

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Background: Strategies for sequencing disease modifying therapies (DMTs) in multiple sclerosis (MS) patients include escalation, high efficacy early, induction, and de-escalation.

Objective: To provide a perspective on de-escalation, which aims to match the ratio of DMT benefit/risk in aging patients.

Methods: We reanalyzed data from a retrospective, real-world cohort of MS patients to model disease activity for oral (dimethyl fumarate and fingolimod) and higher efficacy infusible (natalizumab and rituximab) DMTs by age. For patients with relapsing MS, we conducted a controlled, stratified analysis examining odds of disease activity for oral vs. infusible DMTs in patients <45 or ≥ 45 years. We reviewed the literature to identify DMT risks and predictors of safe discontinuation.

Results: Younger patients had lower probability of disease activity on infusible vs. oral DMTs. There was no statistical difference after age 54.2 years. When dichotomized, patients <45 years on oral DMTs had greater odds of disease activity compared to patients on infusible DMTs, while among those ≥ 45 years, there was no difference. Literature review noted that adverse events increase with aging, notably infections in patients with higher disability and longer DMT duration. Additionally, we identified factors predictive of disease reactivation including age, clinical stability, and MRI activity.

Conclusion: In a real-world cohort of relapsing MS patients, high efficacy DMTs had less benefit with aging but were associated with increased risks. This cohort helps overcome some limitations of trials where older patients were excluded. To better balance benefits/risks, we propose a DMT de-escalation approach for aging MS patients.

Keywords: discontinuation, multiple sclerosis, disease modifying therapy, infection, relapse, high efficacy, de-escalation, escalation

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory disease with associated neurodegeneration affecting the central nervous system (CNS) (1). Over 20 disease modifying therapies (DMTs) are now available that, in both clinical trials and real-world studies, reduce measures of disease activity including new relapses, MRI lesions, and accumulated disability (2). Given differences in efficacy, tolerability, cost, and safety, selection of DMT requires extensive shared decision making between clinicians and patients. These decisions are complicated and should be re-evaluated periodically as relapse and MRI disease activity generally decrease with patient age and duration of disease, likely reflecting a clinical measure of immunosenescence (3). Additionally, the risk of infections and, to a lesser extent, other adverse events frequently increase with age (4–7). Given the number of DMTs and variability of disease activity over the course of a lifetime, there is currently little consensus on the best approaches to treatment.

Here, we will explore what to do later in the treatment of patients with MS and provide our perspective to consider a de-escalation approach. We evaluate the effect of age on disease activity from a recent publication comparing rituximab to natalizumab, fingolimod, and dimethyl fumarate in a real-world setting of 1,246 patients (8). We apply lessons learned from the risk of rituximab in the real-world setting of 1,000 patients and other literature to better inform our perspective on de-escalation of therapy in the treatment of patients with MS (9). Additionally, we review the literature to identify factors associated with disease activity in DMT discontinuation studies to inform a perspective on how to use de-escalation as a treatment strategy.

METHODS

Our analysis utilized data collected for a prior retrospective observational study including participants who had an MS diagnosis; initiated rituximab, natalizumab, fingolimod, or dimethyl fumarate at the Rocky Mountain MS Center at the University of Colorado between January 2010 and October 2013; and, for natalizumab patients only, had a negative JCV serology test at baseline. Detailed methodology and study sample characteristics have previously been reported (8).

We dichotomized patients into two exposure groups defined as either receiving oral DMT or infusible DMT. Our binomial outcome was a composite effectiveness measure defined as the patient experiencing either a clinical relapse, a contrasting enhancing lesion, and/or a new T2 lesion on follow-up MRI within 2 years of drug initiation and while on treatment. The data was then modeled with generalized additive models for our binomial outcome, and penalized cubic regression smoothing splines for the effect of age for the entire cohort and separately by type of DMT (oral or infusible). Using this modeling approach, the age for which 95% confidence intervals overlap indicating no significant difference between groups was identified.

Additionally, for patients with relapsing forms of MS, we conducted a stratified analysis examining odds of disease activity for those on oral vs. infusible DMTs among patients <45 or ≥45 years of age. For this subgroup analysis, three models were used, including simple logistic regression, adjusted logistic regression, and logistic regression on sample group 1:1 nearest neighbor matched by propensity scores (PS) with replacement additionally controlling for covariates. Adjustment methods controlled for age, disease duration, sex, contrast enhancement on baseline MRI, and baseline disease burden (mild, moderate, severe, missing). As a sensitivity analysis, we also examined the outcome of clinical relapse individually using simple logistic regression and adjusted logistic regression.

RESULTS

Our study included a total of 1,246 participants composed of 613 patients on oral DMTs (271 fingolimod, 342 dimethyl fumarate) and 633 patients on infusible DMTs (182 rituximab, 451 natalizumab). **Figures 1A,B** demonstrate the probability of experiencing disease activity within 2 years of drug initiation by age at time of drug initiation for the entire cohort and separated by type of therapy, respectively. When examining the probability of disease activity by type of therapy, there is a statistically significant difference between oral and infusible DMTs up until the age of 54.2, when confidence intervals begin to overlap.

Stratified analyses of patients with relapsing forms of MS included a total of 625 (276 oral, 349 infusible) patients <45 years of age and 379 (233 oral, 146 infusible) patients ≥45 years of age. Baseline characteristics are listed in **Table 1**. Among those <45 years of age, patients on oral DMTs had significantly greater odds of disease activity compared to patients on infusible DMTs [unadjusted odds ratio (OR), 2.67 (95% confidence interval: 1.89, 3.75), $p < 0.001$; adjusted OR, 2.89 (2.02, 4.13), $p < 0.001$; PS 1:1 nearest neighbor matching and controlling for covariates OR, 2.18 (1.34, 3.53), $p = 0.002$]. Among those ≥45 years of age, patients on oral DMTs had no significant difference in odds of disease activity compared to patients on infusible DMTs [unadjusted OR, 1.60 (0.96, 2.64), $p = 0.069$; adjusted OR, 1.65 (0.99, 2.76), $p = 0.053$; PS 1:1 nearest neighbor matching and controlling for covariates OR, 1.16 (0.59, 2.27), $p = 0.675$] (**Supplementary Table 1**). When examining clinical relapses individually, results were consistent. Among those <45 years of age, patients on oral therapies had significantly greater odds of relapse than patents on infusible DMTs [unadjusted OR, 2.82 (1.64, 4.83), $p < 0.001$; adjusted OR, 2.93 (1.71, 5.14), $p < 0.001$]. There was no significant difference in clinical relapses between oral and infusible among those ≥45 years of age [unadjusted OR, 1.27 (0.56, 2.92), $p = 0.567$; adjusted OR, 1.49 (0.67, 3.32), $p = 0.328$].

DISCUSSION

Treatment Strategies

Studies of treatment strategies have primarily focused on choice of DMT early in the disease course, and switching DMT, typically due to intolerance or perceived loss of effectiveness. How to best sequence DMTs and for how long to treat patients with a DMT remain fundamental questions for which there is currently limited evidence. Strategies for sequencing include an escalation

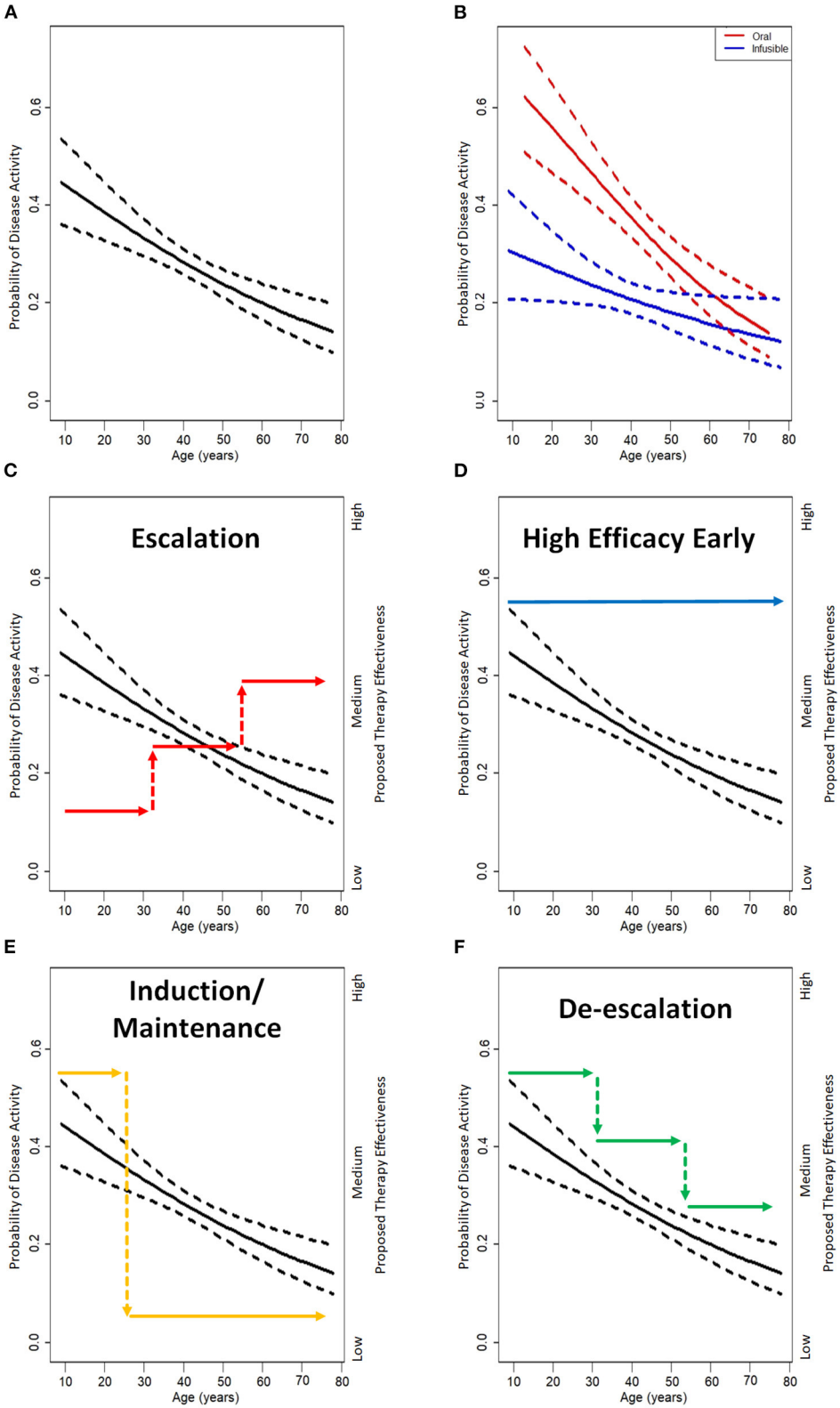

FIGURE 1 | The probability of disease activity decreases over the lifetime of a patient with MS. **(A)** Probability of disease activity (clinical relapse, new T2 lesions, or enhancing lesions) observed in real-world study of 1,246 patients with MS with 95% confidence interval shown in dashed lines. **(B)** The probably of disease activity is

(Continued)

FIGURE 1 | higher in patients on oral (red; dimethyl fumarate and fingolimod) disease mofifying therapies (DMTs) than on infusible (blue; natalizumab and rituximab) DMTs. This probability is higher in younger patients and becomes non-significant by 54.2 years of age where the confidence intervals overlap. **(C)** Given the variable probability of disease activity, it is possible to observe that an escalation treatment approach under-treats early and over-treats as patients age resulting in possibly taking on higher risks but receiving little additional benefit from higher efficacy therapies. **(D)** High efficacy therapy early in the disease course matches the higher probability of disease activity early but can over-treat later in life. **(E)** Induction is often enough to sustain good efficacy early but some patients may breakthrough over time resulting in the need of retreatment or a maintenance therapy. **(F)** A de-escalation approach matches disease activity over a lifetime best, but will benefit from the use of better biomarkers to more rationally prompt changes in DMT. Shared decision making remains a crucial component of deciding on treatment approaches.

TABLE 1 | Baseline characteristics for oral vs. infusible in patients with relapsing forms of MS.

	Oral ($n = 509$)		Infusible ($n = 495$)		*p*-Values
	***n* or mean**	**% or SD**	***n* or mean**	**% or SD**	
Disease duration (years, SD)	10.1	6.5	10.7	6.9	0.215
Age (years, SD)	42.5	11.6	38.6	11.5	<0.001
Gender—Female	370	72.7%	378	76.4%	0.182
Previous DMT*					<0.001
Interferons	74	14.5%	84	17.0%	
Glatiramer acetate	137	26.9%	150	30.3%	
Natalizumab	145	28.5%	60	12.1%	
Rituximab	6	1.2%	0	0.0%	
Fingolimod	17	3.3%	23	4.7%	
Dimethyl fumarate	1	0.2%	2	0.4%	
None	121	23.8%	165	33.3%	
Other	8	1.6%	11	2.1%	
Contrast enhancement on baseline MRI	90	20.4%	143	34.1%	<0.001
Disease burden on baseline MRI					0.629
Mild	232	45.6%	208	42.0%	
Moderate	141	27.7%	152	30.7%	
Severe	53	10.4%	49	9.9%	
Missing	83	16.3%	86	17.4%	

DMT, disease modifying therapy; SD, standard deviation; n, sample size.
**Within 6 months prior to starting study drug.*

approach in which less potent DMTs (e.g., glatiramer acetate, beta-interferons, teriflunomide, S1P receptor modulators, or fumarates) are used first, with transition to more potent therapies if there is evidence of inadequacy (**Figure 1C**). An additional strategy is early use of high efficacy DMTs (e.g., B-cell depletion, natalizumab; **Figure 1D**).

There is increasing evidence that starting with high efficacy DMTs early in the disease course can result in better long-term outcomes compared to the escalation approach (10–12). This concept of treatment escalation is common in medicine. Proponents of an escalation strategy in MS argue that disease activity can be detected early enough for treatment to be effectively escalated and that any damage caused by this breakthrough disease activity is offset by the lower risks of these less potent DMTs. In neurological conditions such as MS, however, the concern is that this inflammation results in permanent damage to the CNS. In addition, current techniques to evaluate disease activity (including markers of progressive disease) are inadequate, and there is likely "silent" worsening separate from overt relapses that may be amenable to intervention and better treated with more effective DMT. Two trials are underway to further evaluate escalation and early high efficacy treatment—Effectiveness of earLy Intensive Vs. Escalation approaches for the Treatment of Relapsing-remitting Multiple Sclerosis (DELIVER-MS, NCT03535298) and Traditional Vs. Early Aggressive Therapy for Multiple Sclerosis Trial (TREAT-MS, NCT03500328). The issue of duration of use for either escalation or early high efficacy therapy is not addressed in these relatively short studies.

A third, less common, approach is use of induction therapies (**Figure 1E**), such as with alemtuzumab and cladribine. These medications are used infrequently, possibly because they are associated with higher perceived risks of infections, cancer, and/or autoimmunity. Autologous hematopoietic stem cell transplant has been used in small numbers of younger patients with highly active disease. All these approaches offer the possibility of immunosuppression that may be long-lasting or even permanent, but patients treated with induction approaches can go on to have disease activity long term which may require retreatment or a maintenance therapy. Thus, durability of these approaches, and duration of use of secondary therapies after their use remains unclear.

Variable Therapy Effectiveness Over a Lifetime

Multiple sclerosis (MS) has inflammatory and degenerative components and, while the mechanisms and timing of transition to progressive disease course are often uncertain, aging plays a key role in both the accumulation of disability and the relative contribution from active inflammation (13). As all FDA-approved MS DMTs are immunosuppressive or immunomodulatory, this has led to assessment of therapies via phase III trials primarily in the population <55 years, when MS is typically most active. While there are substantial individual differences in disease activity across patients, DMTs will generally be most valuable early in the disease course and in younger patients. However, MS is most prevalent in patients in their late 50s, and many of these patients continue DMT (14, 15). Cohort studies indicate that relapse rates are age and time-dependent, with time measured from diagnosis; one study encompassing 2,477 patients over 20 years mean follow-up, relapse rate was proposed to decline by 17% every 5 years with this decline accelerating with age (16). This clinical data is supported by pathology data demonstrating that the rates of active plaques (correlating to enhancing lesions on MRI) decline with age and disease duration (17). Multiple phase III trials of DMTs currently approved for MS have demonstrated a higher efficacy in subgroup analyses of younger patients (18–22). This is supported by a modeling study of >28,000 MS patients treated with 13 types of DMT (ranging from interferons to ocrelizumab and siponimod) in clinical trials demonstrating that high efficacy DMTs do have improved efficacy over other DMTs in patients <40.5 years, but that DMTs generally provide no benefit >53 years (23). It is important to remember that this reflects treatment at the population level as we see that some older patients continue to have disease activity into their 60s or 70s. Our evaluation of an MS population in the real world suggests that high efficacy DMTs still have an additional benefit in patients that are older at least until they are 45 years in binomial models but until they are 54.2 years in linear models. This likely reflects the older population and lack of patients on lower efficacy platform DMTs when compared to the meta-analysis by Weideman et al. (23). Our study is a real-world study and is limited by our ability to control factors such as choosing between treatments and when/where to get MRIs. Therefore, age and disease activity are drivers of outcomes of DMT discontinuation. The Vienna-Innsbruck DMT discontinuation score predicts risk of disease reactivation after discontinuing glatiramer acetate or beta-interferons using a weighted calculation based on age, disease activity on MRI (≥3 new/enlarged T2 lesions or ≥1 gadolinium-enhancing lesion), and duration of stability (years since relapse or EDSS change) (24). In that study, patients age <45 years had a HR 4.3 (2.5, 7.1), $p < 0.001$ and patients age ≥45 and <55 years HR 2.1 (1.4, 3.8), $p < 0.001$, as compared to patients ≥55 years. In addition, activity on MRI <6 months prior to discontinuation had a HR 3.9 (3.2, 4.9), $p < 0.001$, and duration of stability <4 years had a HR 4.4 (2.7, 8.3), $p < 0.001$ and ≥4 and <8 years HR 2.3 (1.6, 4.5), $p < 0.001$, when compared to stability ≥8 years. Ultimately, identification of patients most likely to need ongoing DMT will benefit from multimodal predictive models encompassing radiological, clinical, and other biomarker data, in addition to age and other demographic factors.

Variable Therapy Risks Over a Lifetime

In addition to concerns related to decreased therapeutic impact from DMT with aging and duration of disease, there are substantial concerns that aging increases risks of DMT use. The most pronounced concern is risk of severe infection and the direct relationship of this risk with aging and disability. Multiple sclerosis patients are at increased risk of infections, particularly infections of the respiratory tract and urinary tract (4). Multiple sclerosis patients are also at increased risk for hospitalization from infections (i.e., severe infections) when compared to patients with rheumatological conditions, likely underscoring the impact of accumulated disability from MS (25). In the context of accumulated disability from MS and overall reduced lymphocyte production because of immunosenescence, infection risk increases with age and immunosuppressive DMT (either through impaired trafficking, cellular depletion, or hypogammaglobulinemia depending on DMT mechanism).

Overall serious infections (resulting in hospitalization) in a nationwide Swedish cohort were higher for rituximab (HR 1.7 compared to beta-interferons or glatiramer acetate) than for other DMTs (26). This study found that age needed to be accounted for in these comparisons as infection risk increased with age, as well as disability, lymphopenia, hypogammaglobulinemia, and treatment duration. We evaluated these factors recently in multivariate models on 1,000 patients on long-term rituximab (9). We verified that all of these factors contributed to infections including male gender [OR 2.16 (1.24, 3.77)], rituximab treatment duration [OR 1.33 (1.17, 1.51)], and prior immunosuppression [OR 2.41 (1.19, 4.86)], but disability carried the most weight using stepwise selection models. However, disability had the largest effect of increasing the risk of serious infections [bilateral support (walker) OR 3.14 (1.34. 7.37); wheelchair OR 8.56 (4.47, 16.39)]. Hypogammaglobulinemia is a well-known adverse effect of anti-CD20 therapies and is associated with duration of use of B-cell depleting agents; low IgM may occur in up to 31% of patients and low IgG in up to 7% at 6 years (27).

Progressive multifocal leukoencephalopathy (PML) due to JC virus infection is a complication of treatment with multiple DMTs, most frequently natalizumab. In a 238-patient cohort study of natalizumab-treated patients who developed PML, age >50 years was associated with earlier onset (28). Further, age is associated with increased mortality from PML in the setting of natalizumab (5). Duration of natalizumab treatment is also a pronounced risk factor (29). Cases of PML, some with carry-over from natalizumab, have also been reported with other DMTs (notably with dimethyl fumarate and fingolimod) and may be more common in older patients (30–33). Other infectious complications are also related to aging and duration of treatment. Fingolimod-associated cryptococcal meningitis may be more frequent in older patients and those with treatment duration >2 years (34). Herpes zoster is also reported more frequently

with older age across multiple DMTs (35). Concern regarding infections extends to the effects of COVID-19, for which there is additive risk for poor outcomes with anti-CD20 DMT (36, 37). The COVID-19 pandemic has also heightened the focus on how certain DMTs may reduce the effectiveness of COVID-19 vaccinations (38, 39). The VELOCE trial of ocrelizumab extends this concern to additional vaccines (40).

In addition, malignancy and other agent-specific adverse events are reported in some cases (6, 7). In a meta-regression of 45 trials utilizing DMTs with a variety of mechanisms, depletive DMTs (ocrelizumab and alemtuzumab) were associated with higher incidence of neoplasms, with an effect in those >45 years (41). While there was initial concern based on phase III trial data that ocrelizumab may lead to increased rates of breast cancer, this has not been demonstrated in an analysis of 11 clinical trials and post-marketing surveillance (27). Basal cell carcinoma, for which risk increases with age, has been reported with fingolimod (42, 43). Fingolimod-associated macular edema has also been more common in patients >41 years (44). Taken together, this suggest that non-infectious adverse events increase with age, but this data is not robust.

De-escalation as a Treatment Strategy—Finding a Balance

The natural history of MS changes with aging, with less relapses and MRI disease activity. DMTs have little demonstrated benefit in progressive neurological dysfunction independent of relapses, especially in older patients. In addition, DMT safety may diminish with age, primarily due to increased risk of infections. Finally, most of the phase III trials resulting in approval of MS DMTs have been done in individuals <55 years, yet almost half of adults with MS are ≥55 years, meaning there is minimal safety and efficacy data in older patients with MS. Thus, whether it is necessary and safe to continue DMT as people age remains unclear. Observational studies suggest DMT may be able to be stopped safely later in life, but conditions and timing under which this may be done safely, with minimal risk of recurrent disease activity, remain unclear (45). To better understand these risks, two randomized, controlled, discontinuation trials are looking at discontinuing DMTs in older patients with MS. Discontinuation of DMTs in MS (DISCOMS, NCT03073603) is evaluating stopping DMTs in MS patients of all phenotypes who have been clinically stable for 5 years (radiologically stable for 3 years) and are ≥55 years. A second study looks at DMT Withdrawal in Inactive Secondary Progressive MS Patients Older Than 50 Years (STOP-I-SEP, NCT03653273) and requires clinical and radiological stability for 3 years. A third trial will examine discontinuing treatment in patients as young as 18 years but who have been stable clinically and radiologically for 5 years, Discontinuing DMT in Stable Relapsing-Onset MS (DOT-MS, NCT04260711).

Escalation, induction, and early high efficacy approaches may all be succeeded by ultimate DMT discontinuation over time, and risks of their use may not match with their potential benefits over the age spectrum of MS. As such, de-escalation (**Figure 1F**) is an extension of these emerging strategies that aims to match the potency of DMT with disease activity based on the natural history of MS and, aspirationally, biomarkers for disease activity. This may consist of extended interval or reduced dosing, or potentially transition to a less potent DMT.

De-escalations studies have often involved coming off natalizumab due to concerns with developing PML and include studies about switching to fingolimod and dimethyl fumarate (46, 47). These trials demonstrated that patients with shorter transition times did better and while there is a trend favoring conducting this transition in older patients (≥55 years) this was not adjusted for transition times (47). Though data for extended interval dosing and/or reduced dosing of high efficacy DMT is evolving, these strategies may result in preserved efficacy and reduced risk of adverse effects, in addition to economic advantages. Ultimately, de-escalation strategies according to the data presented above, appear to best match disease activity and deserve to be studied in better detail using randomized controlled trials optimized by use of personalized biomarkers for disease activity, as well as clinical and radiographic monitoring for relapse and disability.

Therefore, we propose a de-escalation treatment approach for patients with MS. High efficacy DMTs are disproportionately more efficacious early in the disease course arguing for early use. This also takes advantage of the relatively low concern for adverse events as these patients are typically younger and have low levels of disability. For these reasons, the benefit of high efficacy DMT is front-loaded. Due to decreasing DMT efficacy and increasing risks, as patients approach 40–55 years, de-escalating should be contemplated. In addition to age, disability (especially in patients who require bilateral support or are wheelchair-bound) should be considered, as should DMT-specific factors that increase infection risk such as JCV seroconversion for natalizumab, lymphocytes <500/μl for dimethyl fumarate, or hypogammaglobulinemia for B-cell depleting therapies. If the patient is clinically stable following de-escalation, discontinuation of DMT may then be an option. This process should be discussed with patients and adjusted based on comfort level and desire for aggressive treatment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Colorado Multiple Institution Review Board (COMIRB) affiliated with the University of Colorado. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors provided substantial contributions to the conception and design of this work, drafting and revising the manuscript critically for important intellectual content, provided approval for publication of the content, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.799138/full#supplementary-material

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Characterization of Antigen-Induced CD4+ T-Cell Senescence in Multiple Sclerosis

*Paula Tomas-Ojer[1], Marco Puthenparampil[1,2], Carolina Cruciani[1], María José Docampo[1], Roland Martin[1] and Mireia Sospedra[1]**

[1] Neuroimmunology and MS Research (NIMS), Department of Neurology, University Hospital and University Zurich, Zurich, Switzerland, [2] Department of Neuroscience DNS, University-Hospital of Padova, Padova, Italy

Antigen-induced T-cell exhaustion and T-cell senescence are peripheral regulatory mechanisms that control effector T-cell responses. Markers of exhaustion and senescence on T Cells indicate the previous activation by repetitive stimulation with specific antigens. Malignant tumors are accompanied by enhanced T-cell exhaustion and T-cell senescence resulting in immune evasion, while these control mechanisms might be diminished in autoimmune diseases including multiple sclerosis (MS). To better understand the involvement of antigen-induced T-cell senescence in controlling CD4+ T-cell-mediated autoimmune responses in MS, we have analyzed the re-expression of CD45RA and the downregulation of CD28 and CD27 molecules as markers of antigen-induced T-cell senescence in fresh cerebrospinal fluid (CSF)-infiltrating and paired circulating T cells from patients with MS. Patients with different levels of CD4+ T-cell senescence were identified and characterized regarding demographical and clinical features as well as intrathecal markers of neurodegeneration. CD4+ T-cell senescence was also analyzed in control patients to explore a putative deficit of this regulatory mechanism in MS. This study shows heterogeneity of markers of CD4+ T-cell senescence in patients with MS. Patients with high levels of CD4+ T-cell senescence in peripheral blood showed increased frequencies of CSF-infiltrating CD28+ CD27-EM CD4+ T cells with a proinflammatory Th1 functional phenotype. The correlation of these cells with the intrathecal levels of neurofilament light chain, a marker of neurodegeneration, suggests their relevance in disease pathogenesis and the involvement of T-cell senescence in their regulation. Markers of antigen-induced T-senescence, therefore, show promise as a tool to identify pathogenic CD4+ T cells in patients with MS.

Keywords: multiple sclerosis (MS), CD4, T-cell, T-cell senescence, regulation

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***Correspondence:**
Mireia Sospedra
mireia.sospedraramos@usz.ch

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INTRODUCTION

T cells are crucial elements of the adaptive immune system to protect us from pathogens and tumors. The potent effector functions of T cells assure protection, but also can represent a risk for self-tissues, if they are not tightly regulated (1). The mechanisms involved in T-cell control include a negative selection in the thymus that prevents the differentiation of T cells with strong reactivity against autoantigens and several peripheral mechanisms that restrain the magnitude and timing

of T-cell responses. These regulatory mechanisms can be T-cell intrinsic because they act directly on the responding T cells and T-cell extrinsic because they depend on other cell subsets, such as regulatory T cells. The T-cell-intrinsic regulatory mechanisms, or checkpoints, control all the T-cell differentiation stages. In naïve T cells, tolerance is maintained by quiescence and ignorance as well as by anergy induced by deficient costimulation during T-cell activation. In effector T cells, the main peripheral tolerance checkpoints are T-cell exhaustion and T-cell senescence induced by repetitive antigen stimulation. Antigen-induced exhausted T cells display reduced responses to antigens and are characterized by decreased cytokine production and high expression of inhibitory receptors, such as programmed cell death protein 1 (PD-1). T-cell senescence is a cell stage, in which cells do not divide anymore as a consequence of telomere shortening and/or DNA damage. Antigen-induced senescent T cells are characterized by telomere erosion, re-expression of CD45RA, downregulation of CD28 and CD27 expression, and increased production of proinflammatory cytokines (2). Other factors, such as oxygen species or ionizing radiation can also induce T-cell senescence by damaging DNA, but the role of this telomer-independent T-cell senescence in maintaining peripheral T-cell tolerance is unknown (1).

An excess of antigen-induced T-cell exhaustion and senescence has been associated with chronic infections (3) and the development of tumors (4). Malignant tumors often promote exhaustion of tumor-infiltrating T cells (TILs) *via* the PD-1/PDL-1 pathway, and PD-1 or PDL-1 target therapies have beneficial effects on several tumors. Tumors also induce markers of T-cell senescence in TILs, such as downregulation of CD27 and CD28 costimulatory molecules. These molecules are required for efficient immune response and are necessary for effective PD1-directed therapy (5, 6). Interestingly, PD-1/PDL-1 targeting therapies have been associated with the adverse development of acute autoimmune reactions and the onset of autoimmune diseases (7). In contrast to cancer or chronic infections, autoimmunity might be associated with reduced T-cell exhaustion since the presence of exhausted T cells has been linked to more favorable clinical outcomes in different autoimmune diseases including MS (8–12). MS is an autoimmune disease of the central nervous system (CNS) (13), in which the expression of PDL-1 in brain lesions (14), the association of PD-1 gene polymorphisms with disease progression (15), the downregulation of PD-1/PD-L1 on peripheral blood mononuclear cells (16), and the increased frequency of PD1+ T cells in patients during remission (12) support an involvement of T-cell exhaustion in disease pathogenesis. Regarding T-cell senescence, premature or accelerated aging that includes immune senescence of different cell types has been described in several autoimmune diseases including MS (17–19). However, the involvement of antigen-induced T-cell senescence in MS and other autoimmune diseases remains unclear.

B cells (20), CD8+ T cells (21, 22), and, particularly, autoreactive CD4+ T cells (13) play a central role in pathogenesis of MS. To better understand the involvement of antigen-induced T-cell senescence in controlling CD4+ T-cell-mediated autoimmune responses in MS, we have analyzed the re-expression of CD45RA and the downregulation of CD28 and CD27 as markers of antigen-induced T-cell senescence (23–25) in fresh cerebrospinal fluid (CSF)-infiltrating and paired circulating T cells from patients with MS. Based on this analysis, patients with different levels of CD4+ T-cell senescence were identified and characterized regarding demographical and clinical features and also intrathecal markers of neurodegeneration. A putative weakness in this regulatory mechanism in MS has also been addressed by comparing intrathecal and peripheral CD4+ T-cell senescence in patients with MS and controls affected of other inflammatory and non-inflammatory neurological diseases.

MATERIALS AND METHODS

Patient Material

Cerebrospinal fluid and paired blood samples obtained for diagnostic purposes were collected from 50 untreated patients with MS, 12 control patients affected by other non-inflammatory neurological diseases (ONINDs), and 12 control patients affected by other inflammatory neurological diseases (OINDs). Patient characteristics are shown in **Supplementary Table S1**. All the patients were recruited at the Neuroimmunology and MS Research Section, Neurology Clinic, University Hospital Zurich (USZ). Diagnosis of MS was based on the revised McDonald criteria (26). The study procedures were approved by the Cantonal Ethics Committee of Zurich (EC-No. 2013-0001) and all the patients or relatives signed informed consent.

Flow Cytometric Immunophenotyping

Cerebrospinal fluid infiltrating and paired circulating cells were immunophenotyped using flow cytometry as previously reported (27). In brief, CSF-infiltrating cells (>10,000 cells in the first hour after collection) and blood circulating cells obtained from 800 ml of peripheral blood after lysis of red blood cells (RBCs) using RBC lysis buffer (BioLegend, San Diego, California, USA) were stained with a cocktail of 13 monoclonal antibodies. Samples were acquired in an LSR Fortessa cytometer (BD Biosciences, Franklin Lakes, New Jersey, USA) and analyzed using FACSDiva (BD) and FlowJO (TreeStar Incorporation, Ashland, Oregon, USA) software. Gatting strategy is shown in **Supplementary Figure S1**.

Enzyme-Linked Immunosorbent Assays

The amount of neurofilament light chain (NF-L) and chitinase 3-like 1 (CHI3L1) proteins were quantified in CSF samples using ELISA (Human Diagnostics, Umea, Sweden and MicroVue, Athens, Ohio, USA, respectively) according to the instructions of the manufacturer.

Statistics

To compare more than two variables, we used the Kruskal–Wallis test for non-normally distributed variables. Linear correlation between variables was tested using the Spearman rank correlation coefficient for non-normally distributed variables. The significance level was set at $p < 0.05$.

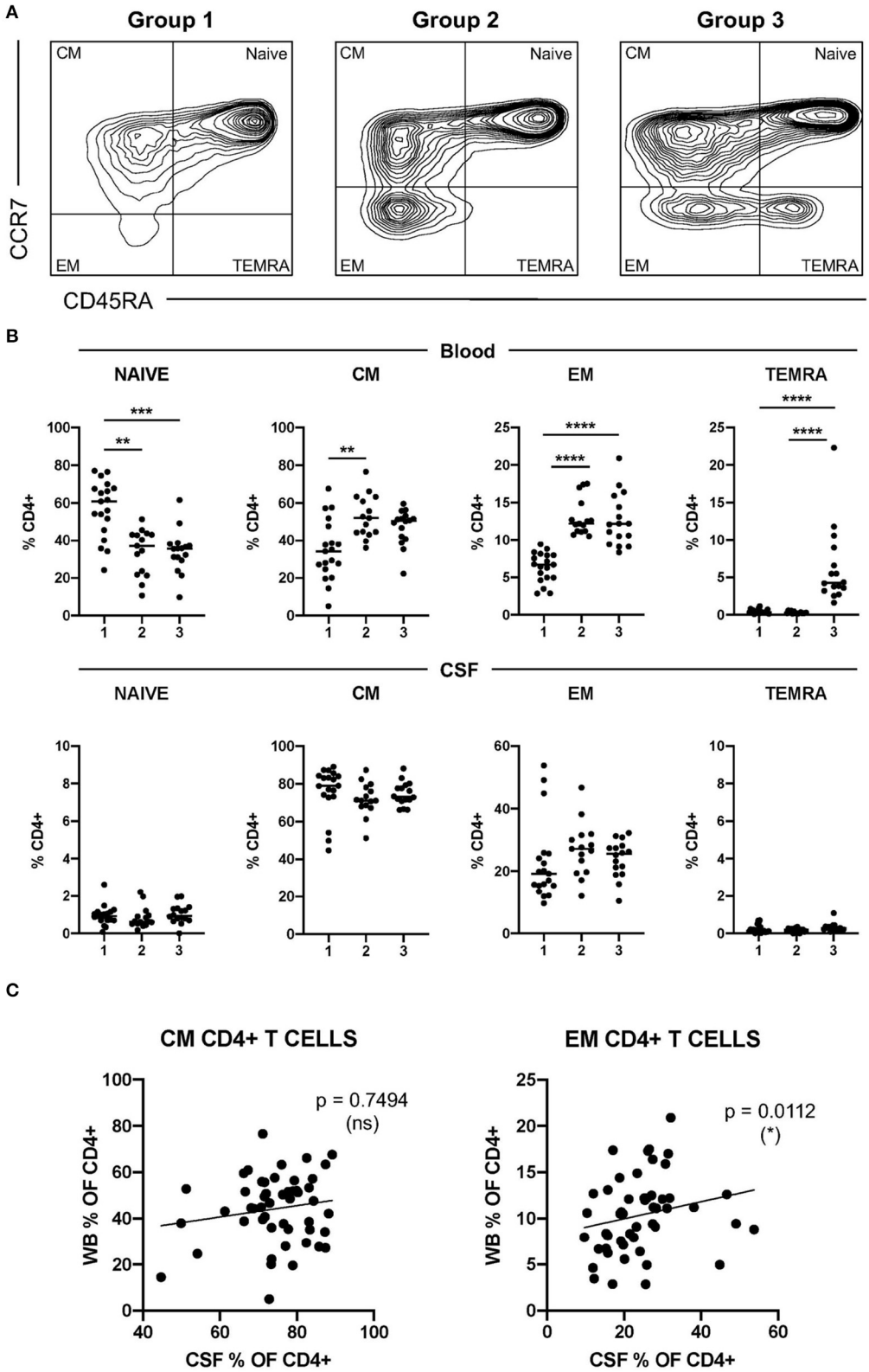

FIGURE 1 | Characterization of CD4+ T-cell senescence using the maturation stage. **(A)** Dot plot showing CCR7 and CD45RA expression on peripheral circulating CD4+ T cells from patients with multiple sclerosis (MS) with low (group 1, left plot), intermediate (group 2, middle plot), and high (group 3, right plot) levels of CD4+

(Continued)

FIGURE 1 | T-cell senescence. **(B)** Frequencies of circulating- and cerebrospinal fluid (CSF)-infiltrating naïve-, CM-, EM-, and terminally differentiated effector memory (TEMRA) CD4+ T cells in patients with MS from groups 1, 2, and 3. **(C)** Correlation between the frequencies in peripheral blood (WB) and CSF of CM- and EM CD4+ T cells from patients with MS. Each dot in the graphs corresponds to a single patient and lines show means. The Kruskal–Wallis test was used to compare patient groups. Linear correlation between variables was tested using Pearson's correlation coefficient. Statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$) is shown.

RESULTS

Characterization of CD4+ T-Cell Senescence Based on the Re-expression of CD45RA

We first identified circulating CD4+ T cells at different maturation stages based on the surface expression of CCR7 and CD45RA [naïve, CCR7+ CR45RA+; central memory (CM), CCR7+ CD45RA-; effector memory (EM), CCR7- CD45RA-; and terminally differentiated effector memory (TEMRA), CCR7- CD45RA+] (**Supplementary Figure S1**). Patients with MS showed a marked heterogeneity regarding the frequencies of circulating CD4+ T cells at the different maturation stages (**Figure 1A**). Based on the assumption that TEMRA CD4+ T cells that re-express CD45RA are the most senescent CD4+ T cells and naïve CD4+ T cells the least senescent, we classified patients with MS into three groups representing low (group 1), intermediate (group 2), and high (group 3) level of CD4+ T-cell senescence (**Figure 1A**). Patients with frequencies of TEMRA CD4+ T cells higher than the mean of all MS patients were classified into group 3. Patients with frequencies of TEMRA CD4+ T cells lower than the mean but with frequencies of EM CD4+ T cells higher than the mean of all patients with MS were classified into group 2. Finally, patients with frequencies of TEMRA- and EM CD4+ T cells lower than the corresponding means were classified into group 1 (**Figure 1A**). Frequencies of circulating naïve, CM, EM, and TEMRA cells in these patient groups are summarized in **Figure 1B**. As expected, group 1 contained significantly higher frequencies of naïve CD4+ T cells than groups 2 and 3, but significantly lower frequencies of EM- and TEMRA CD4+ T cells (**Figure 1B**). We then also analyzed the frequencies of CSF-infiltrating naïve-, CM-, EM- and TEMRA CD4+ T cells in these patient groups (**Figure 1B**). Naïve- and TEMRA CD4+ T cells were practically absent in all CSF samples (**Figure 1B**). The frequencies of CSF-infiltrating CM- and EM CD4+ T cells did not show significant differences between groups (**Figure 1B**). Accordingly, the correlation between the frequencies of circulating and CSF-infiltrating CM- and EM CD4+ T cells was very low or absent (**Figure 1C**).

Characterization of CD4+ T-Cell Senescence Based on the Downregulation of CD28 and CD27

To further characterize CD4+ T-cell senescence in MS, we included the downregulation of CD28 and CD27 molecules in our analysis (**Supplementary Figure S1**). As expected, the downregulation of CD28 and CD27 costimulatory molecules was associated with the maturation stage of circulating CD4+ T cells (**Figure 2A**). The terminally differentiated TEMRA CD4+ T cells contained the highest frequencies of CD28- CD27- cells while naïve- and CM CD4+ T cells contained the highest frequencies of CD28+ CD27+ cells (**Figure 2A**). EM CD4+ T cells showed intermediate frequencies of CD28+ CD27+, CD28+ CD27-, and CD28- CD27- cells, while the frequencies of CD28- CD27+ cells were very low in all the differentiation stages (**Figure 2A**).

Figure 2B shows the downregulation of CD28 and CD27 in circulating and CSF-infiltrating EM CD4+ T cells from the three groups of patients with different levels of CD4+ T-cell senescence in peripheral blood. As expected, patients from group 1 showed significantly higher frequencies of circulating EM CD28+ CD27+ and significantly lower frequencies of circulating EM CD28+ CD27- and CD28- CD27- than patients from group 3 (**Figure 2B**). Interestingly, patients from group 1 also showed significantly higher frequencies of CSF-infiltrating EM CD28+ CD27+ and significantly lower frequencies of CSF-infiltrating EM CD28+ CD27- and CD28- CD27- than patients from group 3 (**Figure 2B**). Accordingly, the frequencies of these cells in peripheral blood and CSF showed strongly significant correlations (**Figure 2C**). The frequencies of circulating and CSF-infiltrating EM CD28- CD27+ CD4+ T cells did not show any differences between the patient groups or a significant correlation between them (**Figures 2B,C**).

Functional Phenotype of CD28+ CD27+ and CD28+ CD27- EM CD4+ T Cells

Using the surface expression of chemokine receptors, we classified circulating and CSF-infiltrating CD28+ CD27+ and CD28+ CD27- EM CD4+ T cells into the following functional phenotypes: Th1 (CCR6- CCR4-), Th2 (CCR6- CCR4+), Th1* (CCR6+ CCR4-) and Th17 (CCR6+ CCR4+) (**Supplementary Figure S1**). The low numbers of CD28- CD27+ and CD28- CD27- EM CD4+ T cells impeded to determine their functional phenotype. The functional phenotype of circulating and CSF-infiltrating CD28+ CD27+ EM CD4+ T cells did not show significant differences between patients with different levels of CD4+ T-cell senescence (**Figure 3**). However, the frequencies of circulating and particularly of CSF-infiltrating CD28+ CD27- EM CD4+ T cells with a Th1 functional phenotype were significantly higher in patients of group 3. These patients also showed significantly lower frequencies of CSF-infiltrating CD28+ CD27- EM CD4+ T cells with a Th2 functional phenotype (**Figure 3**).

We evaluated CNS damage in our patient cohort with MS using neurofilament light chain (NF-L), a promising intrathecal biomarker of neurodegeneration (28) and chitinase 3-like 1 (CHI3L1), a glycoprotein secreted by activated glia (29). The intrathecal amount of NF-L but not of CHI3L1 showed a

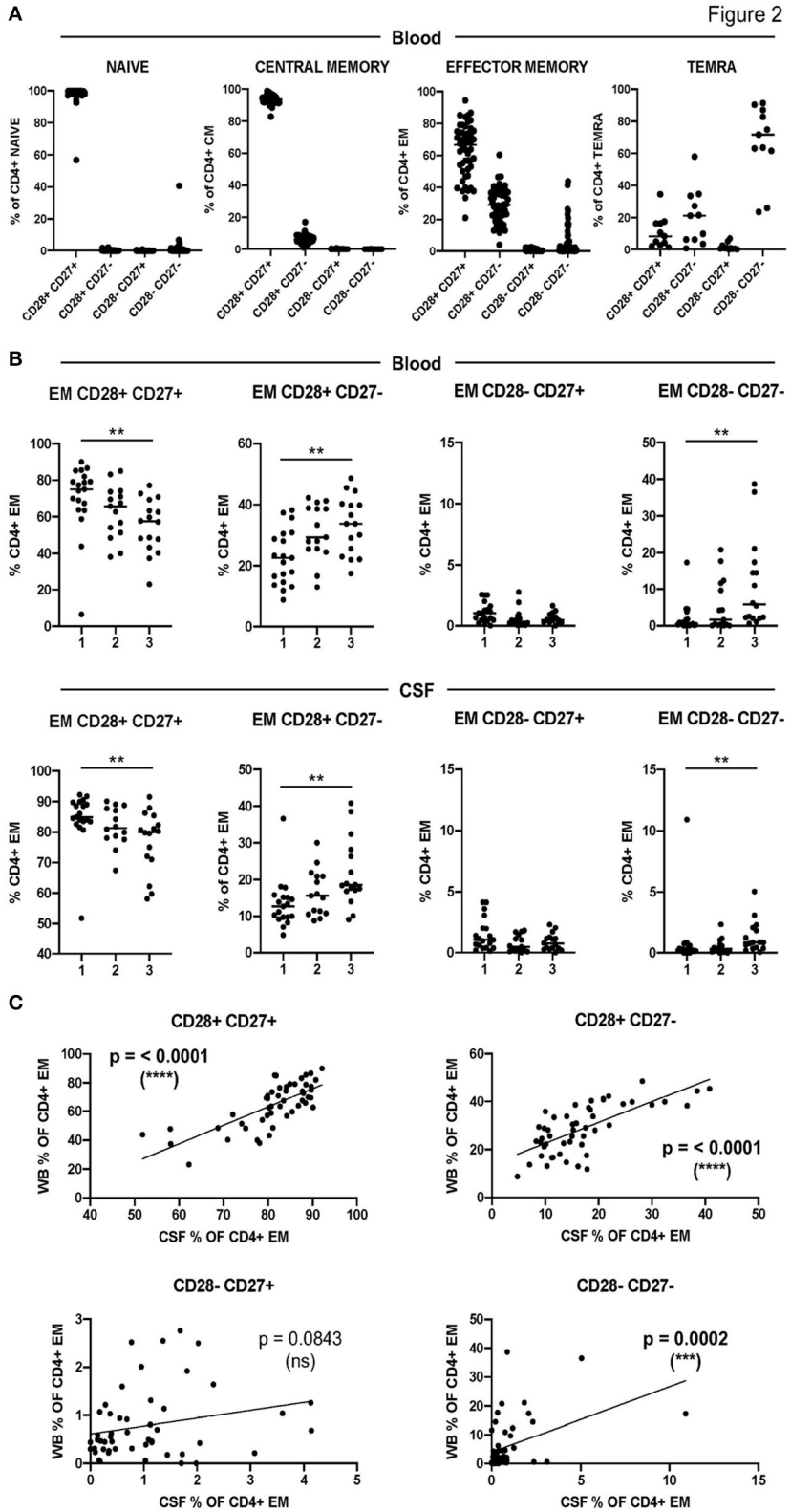

FIGURE 2 | Characterization of CD4+ T-cell senescence using the downregulation of CD28 and CD27. **(A)** Frequencies of CD28+ CD27+, CD28+ CD27-, CD28- CD27+, and CD28- CD27- cells among circulating naïve-, CM-, EM-, and TEMRA CD4+ T cells. **(B)** Frequencies of CD28+ CD27+, CD28+ CD27-, CD28- CD27+, *(Continued)*

FIGURE 2 | and CD28- CD27- cells among circulating (upper graphs) and CSF-infiltrating (lower graphs) EM CD4+ T cells in the three groups of patients with low (1), intermediate (2), and high (3) levels of CD4+ T-cell senescence. **(C)** Correlation between the frequencies in peripheral blood (WB) and CSF of CD28+ CD27+, CD28+ CD27-, CD28- CD27+, and CD28- CD27- EM CD4+ T cells from patients with MS. Each dot in the graphs corresponds to a single patient and lines show means. The Kruskal–Wallis test was used to compare patient groups. Linear correlation between variables was tested using Pearson's correlation coefficient. Statistical significance (**$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$) is shown.

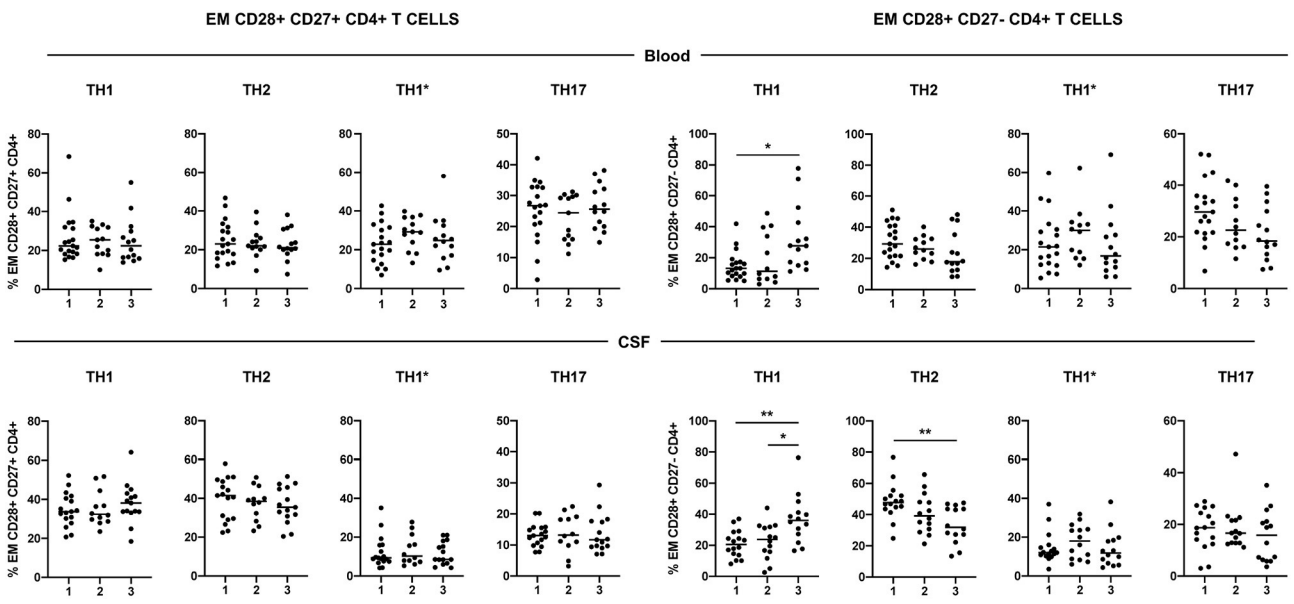

FIGURE 3 | Functional phenotype of CD28+ CD27+ and CD28+ CD27- EM CD4+ T cells. Frequencies of circulating- (upper graphs) and CSF-infiltrating (lower graphs) Th1 (CCR4- CCR6-), Th2 (CCR4+ CCR6-), Th1* (CCR4- CCR6+) and Th17 (CCR4+ CCR6+) EM CD28+ CD27+ (left graphs) and CD28+ CD27- (right graphs) CD4+ T cells. Each dot in the graphs corresponds to a single patient and lines show means. The Kruskal–Wallis test was used to compare patient groups. Statistical significance (*$p < 0.05$ and **$p < 0.01$) is shown.

TABLE 1 | Demographic and clinical features.

		Level of CD4 senescence		
	All	**1**	**2**	**3**
Number of patients	50	19	15	16
Female/male ratio	1.77	2.16	1.14	1.67
Age (years)	36.2 ± 10.6	34.4 ± 8.2	35.8 ± 9.5	38.7 ± 13.9
Disease duration (days)	1044.8 ± 1869.1	1004.5 ± 1315.9	1786.5 ± 1796.7	1363.0 ± 1906.3
Clinical course				
RIS/CIS # (%)	10 (20)	5 (26.3)	4 (33.3)	1 (6.2)
RRMS # (%) in review	36 (72)	14 (73.7)	8 (53.4)	14 (87.6)
PMS* # (%)	4 (8)	0	3 (13.3)	1 (6.2)
DR15 (% patients)	22 (44)	11 (57.8)	6 (40)	5 (31.2)
CSF				
CSF cell count (cells/uL)	7.08 ± 7.7	6.11 ± 3.9	9 ± 12.2	6.53 ± 6.16
BBB damage** (% patients)	11 (22)	4 (21)	2 (13.3)	5 (31.2)
IgG index	1.02 ± 0.7	1.06 ± 0.5	1.12 ± 1.02	0.87 ± 0.48
IgM index	0.15 ± 0.19	0.12 ± 0.13	0.20 ± 0.28	0.16 ± 0.15
IgA index	0.34 ± 0.28	0.41 ± 0.45	0.28 ± 0.04	0.31 ± 0.04

**PMS, secondary progressive MS and primary progressive MS.*

***Blood–brain barrier (BBB) damage (QALB-QNORM > 0).*

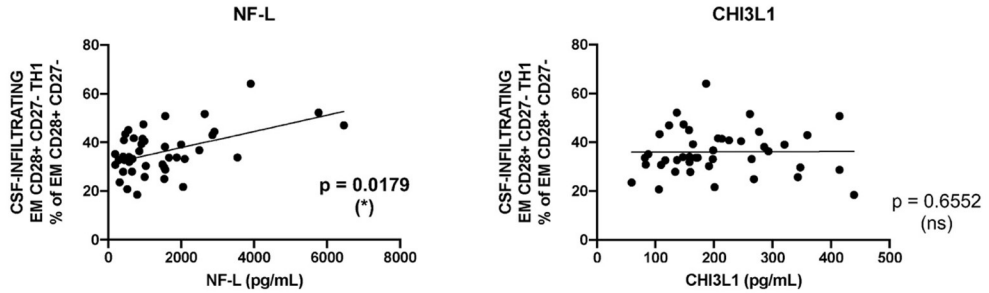

FIGURE 4 | Correlation between CD28+ CD27- EM CD4+ Th1 cells and markers of tissue damage. Correlation between the frequencies of CSF-infiltrating CD28+ CD27- EM CD4+ Th1 cells from patients with MS and the intrathecal amounts of NF-L (left graph) and CHI3L1 (right graph). Each dot in the graphs corresponds to a single patient. Linear correlation between variables was tested using Pearson's correlation coefficient. Statistical significance (*$p < 0.05$) is shown.

significant correlation with the frequencies of CSF-infiltrating EM CD28+ CD27- CD4+ Th1 cells (**Figure 4**).

Characterization of Patients With Different Levels of CD4+ T-Cell Senescence

Next, we compared demographical and clinical features of MS patients with different levels of CD4+ T-cell senescence (**Table 1**). There were no significant differences between patient groups regarding gender, age at the spinal tap, disease duration, clinical course, or the frequency of patients expressing the MS-associated DR15 haplotype. We did not find significant differences neither regarding routine CSF parameters, such as the number of CSF-infiltrating cells, blood-brain barrier (BBB) permeability, or immunoglobulin indices.

Comparison of CD4+ T-Cell Senescence in Patients With MS and Controls

Finally, we compared CD4+ T-cell senescence in patients with MS and controlled affected by ONINDs and OINDs from whom CSF and paired blood samples were available. Patients with MS and patients with OIND showed significantly higher numbers of CSF-infiltrating T cells than patients with ONIND, while only patients with MS showed a significantly higher immunoglobulin G (IgG) index (**Figure 5A**). Although OIND was older, age differences did not reach statistical significance.

The frequencies of circulating naïve CD4+ T cells in patients with MS were significantly higher than in patients with OIND, while the frequencies of CM CD4+ T cells were lower (**Figure 5B**). The younger age of patients with MS might be the reason for these differences, since the frequency of both the cell subtypes in blood correlated with age (**Supplementary Figure S2**). We did not find significant differences between patients with MS and controls for circulating EM- and neither for TEMRA CD4+ T cells (**Figure 5B**). The frequencies of circulating CD28+ CD27+, CD28+ CD27-, CD28- CD27+, and CD28- CD27- CD4+ T cells at the different maturation stages (naïve, CM, EM, and TEMRA) from patients with MS and controls did not show statistically significant differences either (**Figure 1C**).

We further compared CD4+ T cell senescence in freshly isolated CSF-infiltrating CD4+ T cells from patients with MS and controls. Naïve- and TEMRA CD4+ T cells were practically absent in all CSF samples (**Figure 6A**). The frequencies of CM- and EM CD4+ T cells did not show significant differences between patients with MS and controls (**Figure 6A**). We then compared the frequencies of CSF-infiltrating CD28+ CD27+, CD28+ CD27-, CD28- CD27+, and CD28- CD27- EM CD4+ T cells in patients with MS and controls. Only the frequencies of CD28+ CD27+ EM CD4+ T cells were significantly higher in patients with MS compared with ONIND (**Figure 6B**).

DISCUSSION

Antigen-induced T-cell exhaustion and T-cell senescence are considered as important regulatory mechanisms controlling immune responses mediated by effector T cells (1). In malignant tumors, these mechanisms are enhanced and allow the tumor to evade the immune system (4), while they might be reduced in autoimmunity. MS is considered an autoimmune disease of the CNS, in which immune responses mediated by autoreactive CD4+ T cells seem to play a crucial role (13). Inadequate T-cell exhaustion (12, 16) and maybe also T-cell senescence might facilitate these autoreactive responses in patients with MS. With the aim to better understand antigen-induced T-cell senescence in MS and its putative role in disease pathogenesis, we have characterized CD4+ T-cell senescence in patients with MS and controls.

In a first step, we used the surface expression of CCR7 and CD45RA to analyze the maturation stage of circulating CD4+ T cells in patients with MS. At this first level of analysis, we found a high degree of heterogeneity in frequencies of naïve-, CM-, EM-, and TEMRA CD4+ T cells between patients with MS and, therefore, grouped patients into low (group 1), intermediate (group 2), and high (group 3) level of CD4+ T-cell senescence. The analysis of CSF-infiltrating cells demonstrated that mainly CM- and EM CD4+ T cells cross the BBB and that their frequencies in the CSF were not associated with their frequencies in peripheral blood suggesting a selective recruitment of CM- and EM CD4+

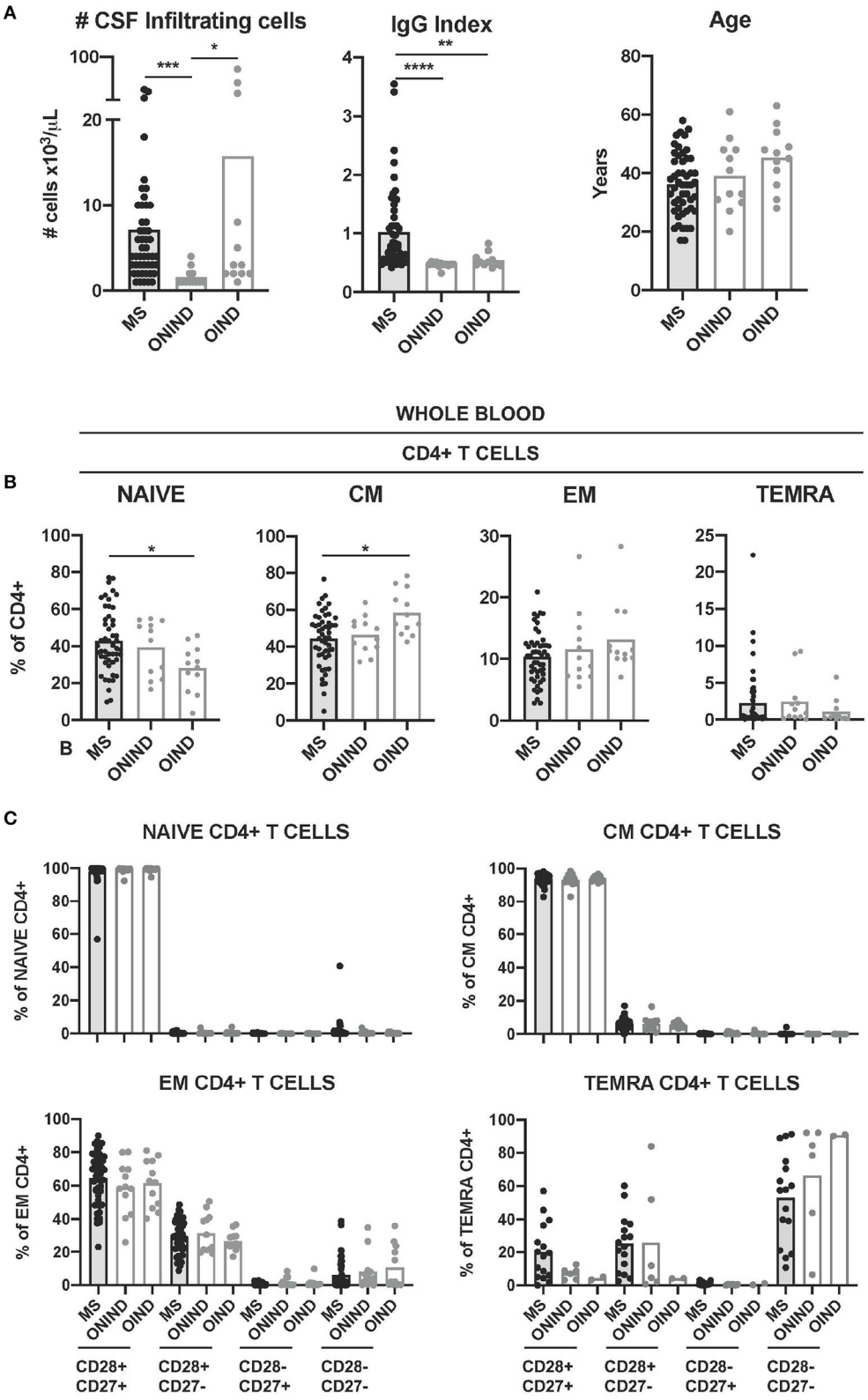

FIGURE 5 | Comparison of circulating CD4+ T-cell senescence between patients with MS and controls. **(A)** Graphs showing the number of CSF-infiltrating cells (left graph), IgG Index (middle graph) and age in years (right graph) in patients with MS ($n = 50$) and control patients affected of other non-inflammatory neurological

(Continued)

FIGURE 5 | disease (ONIND) ($n = 12$) and other inflammatory neurological disease (OIND) ($n = 12$). **(B)** Frequencies of circulating naïve-, CM-, EM-, and TEMRA CD4+ T cells in patients with MS and controls. **(C)** Frequencies of CD28+ CD27+, CD28+ CD27-, CD28- CD27+, and CD28- CD27- cells among circulating naïve-, CM-, EM-, and TEMRA CD4+ T cells from patients with MS and controls. Each dot in the graphs corresponds to a single patient and bars show means. The Kruskal–Wallis test was used to compare patients with MS and controls. Statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$) is shown.

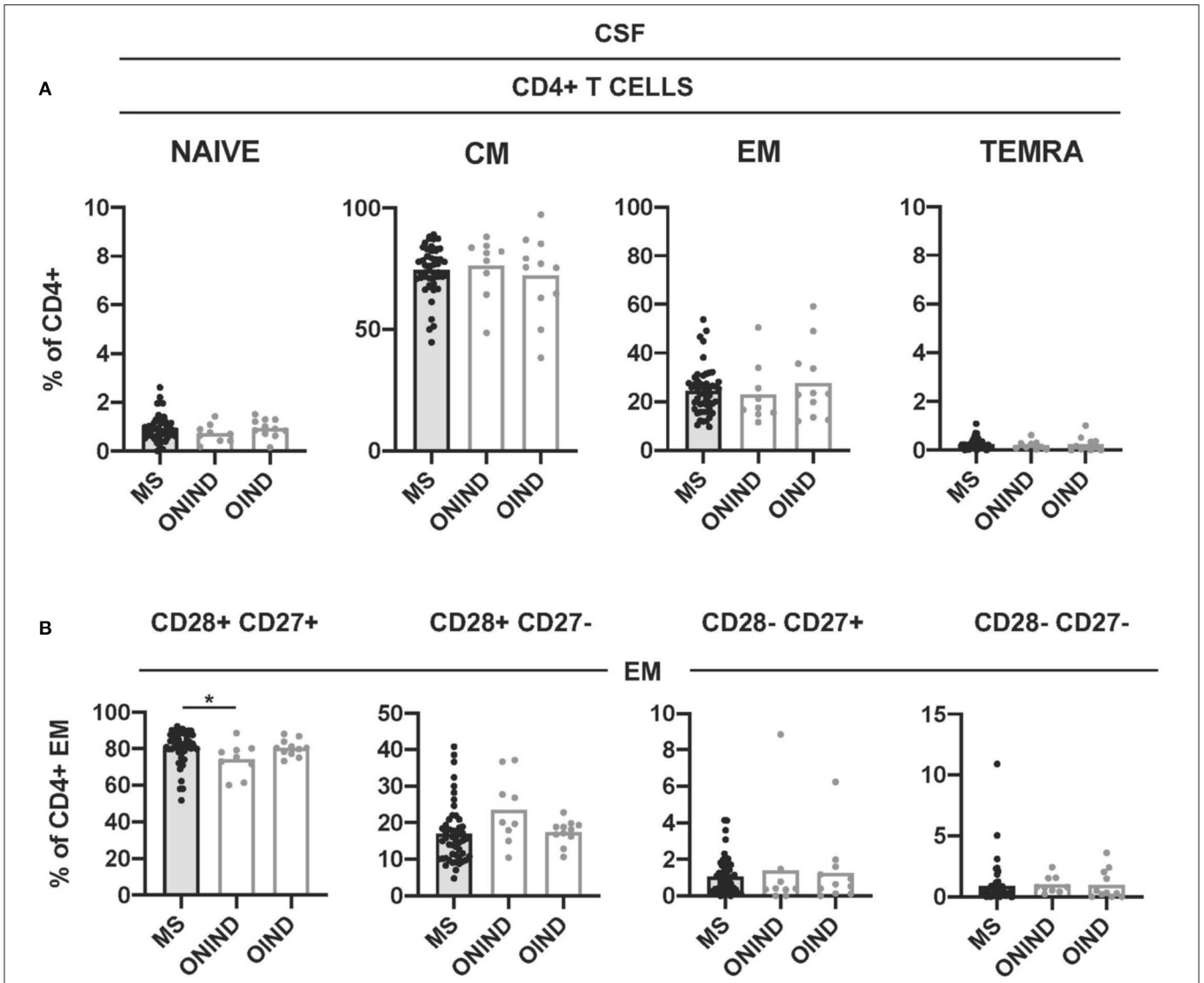

FIGURE 6 | Comparison of intrathecal CD4+ T-cell senescence between patients with MS and controls. **(A)** Frequencies of CSF-infiltrating naïve-, CM-, EM-, and TEMRA CD4+ T cells in patients with MS and controls. **(B)** Frequencies of CD28+ CD27+, CD28+ CD27-, CD28- CD27+, and CD28- CD27- cells among CSF-infiltrating EM CD4+ T cells from patients with MS and controls. Each dot in the graphs corresponds to a single patient and bars show means. The Kruskal–Wallis test was used to compare patients with MS and controls. Statistical significance (*$p < 0.05$) is shown.

T cells into the CNS. By combining the maturation stage and the downregulation of CD28 and CD27 costimulatory molecules, we further defined 16 putative stages of CD4+ T cell senescence in which naïve CD28+ CD27+ cells should be the least and TEMRA CD28- CD27- cells the most senescent T cells. Supporting that this second analysis to evaluate CD4+ T-cell senescence is sound, we found that CD28- CD27- cells were more abundant among circulating TEMRA CD4+ T cells and CD28+ CD27+ among circulating naïve- and CM CD4+ T cells. Circulating EM CD4+ T cells, with an intermediate maturation stage correspondingly showed intermediate levels of CD28+ CD27+ and CD28- CD27- cells. T-cell senescence is considered a regulatory mechanism of effector cells, and accordingly, the downregulation of CD28 and CD27 molecules was found mainly for EM and TEMRA cells. This second analysis confirmed the heterogeneity of the patients and supported our

initial classification into the three groups. Patients from group 1 showed significantly higher frequencies of circulating EM CD28+ CD27+ and lower frequencies of CD28+ CD27- and CD28- CD27- cells than patients from group 3. Interestingly, the frequencies of circulating- and CSF-infiltrating CD28+ CD27+, CD28+ CD27-, and CD28- CD27- EM CD4+ T cells correlated strongly. These results suggest that, while the CD4+ T-cell maturation stage influences migration through the BBB, the downregulation of CD28 and CD27 does not seem to influence this migration.

Circulating- and CSF-infiltrating CD28- CD27+ CD4+ T cells showed comparably low frequencies for all maturation stages. Furthermore, while the frequencies of circulating and CSF-infiltrating CD28+ CD27+, CD28+ CD27-, and CD28- CD27- EM CD4+ T cells were significantly different between groups 1 and 3, the frequencies of circulating- and CSF-infiltrating CD28- CD27+ EM CD4+ T cells were comparable in the three groups of patients. These results suggest that CD28- CD27+ cells most likely do not represent a senescent stage and that the induction of senescence in CD4+ T cells probably always starts with the downregulation of CD27 and only then of CD28.

As mentioned earlier, the induction of T-cell senescence is considered as a regulatory mechanism to control immune responses. We can assume that cells downregulating CD28 and CD27 molecules are cells that underwent more rounds of antigen stimulation *in vivo* and, therefore, are cells that are likely relevant in disease pathogenesis. In this context, the use of markers of T-cell senescence might facilitate the identification of relevant pathogenic T cells and the detailed characterization of these pathogenic T cells including the determination of their specificity is crucial to better understand MS and to develop new therapeutic approaches. Supporting the assumption that markers of T-cell senescence might be useful to identify pathogenic T cells in MS, it is important to note that CD28+ CD27- EM CD4+ T cells with a proinflammatory Th1 functional phenotype that were significantly more frequent in patients of group 3 showed a correlation with the intrathecal amount of NF-L, a biomarker of CNS damage. Interestingly, CD28+ CD27- TEMRA and EM CD4+ T cells expressing Th1- and cytotoxicity-associated genes have also been described to be increased and associated with higher damage in rheumatoid arthritis patients (17, 30). Furthermore, we found that MS patients with intrathecal CD4+ T-cell reactivity against GDP-L-fucose synthase derived peptides and that are characterized by higher neuroinflammation and neurodegeneration, also showed higher frequencies of CD28+ CD27- TEMRA and EM CD4+ T cells expressing Th1- and cytotoxicity-associated genes (31). Altogether these data support a pathogenic role of CD28+ CD27- TEMRA and EM CD4+ T cells in autoimmunity. Furthermore, data supporting a role of Th1 cells in MS are: (i) an increased autoproliferation of CM- and EM CD4+ Th1 cells (32), (ii) higher frequency of myelin basic protein (MBP)-specific Th1+ CD4+ T cells in patients with MS (33), (iii) higher sensitivity of naive CD4+ CD45RA+ to activation by MBP (34), and (iv) involvement of a Th1 axis between T cells and CD11c+ B cells in MS (35).

Due to the dual meaning of markers of T-cell senescence on CD4+ T cells, i.e., regulation vs. the previous activation, it is difficult to discern whether a high level of CD4+ T-cell senescence in some patients with MS reflects better T-cell regulation in these patients or a higher antigen-specific T-cell activation. The fact that patients from group 3 showed higher frequencies of CSF-infiltrating CD28+ CD27- EM CD4+ Th1 cells associated with neurodegeneration, suggests that a high level of CD4+ T-cell senescence most likely reflects higher antigen-specific T-cell activation. In this study, however, we have not been able to associate a high level of CD4+ T-cell senescence with high-disease activity to support this hypothesis. The limited clinical data regarding disability evolution and imaging findings that were available rendered an analysis to associate markers of senescence with the level of disease activity impossible. We think that further research in a large cohort of well-characterized patients is required to determine whether markers of T-cell senescence reflect higher T activation or T-cell regulation and what they mean for understanding T-cell senescence/activation in MS.

Our comparison of patients with MS and controls did not demonstrate a defective or accelerated T-cell senescence in patients with MS compared with controls. Further research in a larger number of control samples should be pursued to clarify putative defects in T-cell senescence in MS.

In summary, our results suggest that T-cell senescence most likely contributes to controlling autoimmune responsiveness in MS. An in-depth characterization of CD4+ T-cell senescence in patients with MS by combining the maturation stage and the downregulation of CD28 and CD27 costimulatory molecules might therefore facilitate a more detailed characterization of pathogenic CD4+ T cells. Further research should be pursued to determine the validity of these markers to identify patients with more aggressive forms of the disease and also to clarify whether T-cell senescence is compromised or not in patients with MS.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Cantonal Ethics Committee of Zurich (EC-No. 2013-0001). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PT-O and MP: major role in the acquisition of data. CC and MD: acquisition of data. RM: interpreted the data and revised the manuscript for intellectual content. MS: design and conceptualized study, analyzed the data, interpreted the data, and drafted the manuscript for intellectual content.

All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.790884/full#supplementary-material

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REVIEW
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Molecular Mechanisms of Immunosenescene and Inflammaging: Relevance to the Immunopathogenesis and Treatment of Multiple Sclerosis

Océane Perdaens[1] *and Vincent van Pesch*[1,2]*

[1] *Laboratory of Neurochemistry, Institute of Neuroscience, Université catholique de Louvain (UCLouvain), Brussels, Belgium,* [2] *Department of Neurology, Cliniques universitaires Saint-Luc, Université catholique de Louvain (UCLouvain), Brussels, Belgium*

Aging is characterized, amongst other features, by a complex process of cellular senescence involving both innate and adaptive immunity, called immunosenescence and associated to inflammaging, a low-grade chronic inflammation. Both processes fuel each other and partially explain increasing incidence of cancers, infections, age-related autoimmunity, and vascular disease as well as a reduced response to vaccination. Multiple sclerosis (MS) is a lifelong disease, for which considerable progress in disease-modifying therapies (DMTs) and management has improved long-term survival. However, disability progression, increasing with age and disease duration, remains. Neurologists are now involved in caring for elderly MS patients, with increasing comorbidities. Aging of the immune system therefore has relevant implications for MS pathogenesis, response to DMTs and the risks mediated by these treatments. We propose to review current evidence regarding markers and molecular mechanisms of immunosenescence and their relevance to understanding MS pathogenesis. We will focus on age-related changes in the innate and adaptive immune system in MS and other auto-immune diseases, such as systemic lupus erythematosus and rheumatoid arthritis. The consequences of these immune changes on MS pathology, in interaction with the intrinsic aging process of central nervous system resident cells will be discussed. Finally, the impact of immunosenescence on disease evolution and on the safety and efficacy of current DMTs will be presented.

Keywords: multiple sclerosis, immunosenescence, inflammaging, T/B cells, oligodendrocytes, microglia, astrocytes, disease modifying therapies

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Niels Hellings,
University of Hasselt, Belgium
Mirjana Dimitrijević,
University of Belgrade, Serbia

***Correspondence:**
Vincent van Pesch
vincent.vanpesch@saintluc.uclouvain.be

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INTRODUCTION

With the aging of the world population, seniors aged over 65 years, that account for 9.3% of the global population in 2020, are predicted to have doubled in absolute number by 2050, representing 15.9% (1). This increase in life expectancy inevitably has an impact on disease prevalence and incidence, especially of chronic diseases. Health care systems worldwide must face this demographic evolution within the next decades. Biological aging is the decline in homeostasis, with functional alterations of all organs and tissues, resulting in an increase

of morbidity and mortality (2). On a cellular level, senescent cells, accumulating with age, are arrested in their cell cycle, but are still active, although functionally dysregulated and affecting their microenvironment by secreting soluble signaling factors (interleukins, chemokines, growth factors), proteases, or insoluble proteins/extracellular components. These constitute the so-called senescence-associated secretory phenotype (SASP) exerting a paracrine pro-inflammatory effect (3, 4). The immune system, which is continuously operating throughout life, is prone to these age-related changes, referred to as immunosenescence (5). Immunosenescence affects both the innate, and, to a greater extent, the adaptive immunity. It is postulated to explain increased prevalence of infections, cancers and auto-immune diseases and reduced response to vaccination in the elderly (6). On the contrary, the purpose of cell cycle arrest in senescent cells is to prevent cellular escape into tumoral processes (7). However, no single immune change associated with senescence explains health-related consequences of aging. Hence, a longitudinal study proposes an age-related 'immune risk phenotype' associated with poorer survival, characterized by an inversion in the $CD4^+/CD8^+$ T cell ratio, the expansion of the terminally differentiated $CD8^+CD28^-$ T cells, lower B cell numbers and seroconversion for cytomegalovirus (CMV) (8–10). Moreover, a chronic, sterile low-grade inflammation occurs concurrently, named inflammaging, mutually interacting with immunosenescence. Continuous antigenic load and stressors stimulate the innate immune system, mainly macrophages, to produce pro-inflammatory cytokines, such as interleukin (IL) 1, IL6, or tumor necrosis factor (TNF), also part of the SASP (11). However, centenarians aging healthily have an inverted immune risk phenotype and a heightened inflammaging profile properly counterbalanced by anti-inflammaging (12–14). Hence, Franceschi et al. argument that diseases arise when this equilibrium is broken (11, 15).

The multiple sclerosis (MS) population older than 65 years is increasing worldwide due to improving life expectancy with MS, although the latter remains 6–10 years shorter as compared to the general population (16, 17). There is growing awareness about the implications of aging with MS, due to immunosenescence, the high burden of comorbidities and the lack of knowledge on long-term effects of exposure to disease modifying therapies (DMTs). The safety, efficacy and benefit of DMTs in this population are unknown, since patients over 55-to-60-years-old are generally excluded from clinical trials (17). Furthermore, while remitting-relapsing MS (RRMS) is the prominent phenotype in younger patients, older patients more likely have primary or secondary progressive MS (PPMS/SPMS), in which chronic inflammation and neurodegeneration, due to failure in myelin repair and axonal loss, is considered to prevail (18). However, the pathophysiology underlying the progression of the disease with aging remains incompletely understood.

We aim to review and compare current knowledge on immunosenescence and inflammaging, relative to MS and other autoimmune diseases (AIDs), such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), as these AIDs are amongst the most studied in this context (19–21). In the setting of MS, we will also focus on the concomitant senescence of central nervous system (CNS) resident cells in order to answer several questions. (a) What is the evidence or the lack thereof to consider MS a disease of premature immunosenescence? (b) What are the common or different immunosenescence features found in MS and other autoimmune diseases? (c) Are epigenetic changes involved in immunosenescence? (d) Is the age-related evolution of MS toward a progressive phenotype linked to immunosenescence? (e) Does immunosenescence expose aging MS patients to increased risks of infection and cancer, especially when taking DMTs?

GENERAL MECHANISMS OF IMMUNOSENESCENCE

Immunosenescence is defined as the physiological aging of the immune system (22). Immune cells are generated from hematopoietic stem cells (HSCs) throughout life and differentiate stepwise, undergoing selection and proliferation pressure upon antigenic contact. They are thus especially prone to senescent processes. Changes related to immunosenescence are more preeminent within the adaptive than the innate immune system [reviewed by (23, 24)].

Cell cycle arrest of aging cells is initially a protective phenomenon against increasing cellular damage and tumorigenesis (7). The pro-inflammatory SASP (IL6, IL8, matrix metalloproteinase (MMP) 1/3, monocyte chemotactic protein (MCP) 2/3, insulin growth factor binding proteins) of senescent cells constitutes a removal-signal directed toward immune cells (4). However, due to age-related dysfunctions in the immune system, this clearance partly fails (25). The pro-inflammatory and oxidative context occurring during the aging process, enhances the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway, a key regulator of inflammation (26). This compensatory mechanism may become self-deleterious, since cumulative cell debris, self-antigens and the inflammatory SASP contribute together to inflammaging, altering cell, tissue, organ and organism homeostasis [reviewed by (23)].

Immunosenescence has been implicated in reduced defenses against infections and reduced response to vaccination (due to a reduced antigenic response by T and B cells), an increased risk of

Abbreviations: ABC, age-associated B; AID, autoimmune disease; BBB, blood brain barrier; BCR, B cell receptor; CMV, cytomegalovirus; CNS, central nervous system; CSF, cerebrospinal fluid; DC, dendritic cell; DMT, disease-modifying therapy; EAE, experimental autoimmune encephalomyelitis; EBV, Epstein Barr Virus; FLS, fibroblast-like synoviocyte; HLA-DR, Human Leukocyte Antigen-DR isotype; HSC, hematopoietic stem cell; Ig, immunoglobulin; IFN-I, type I interferon; KREC, K-deleting recombination excision circles; MHC-I/II, major histocompatibility complex of class I or II; miRNA, microRNA; MS, multiple sclerosis; mtDNA, mitochondrial DNA; NET, neutrophil extracellular trap; NK, natural killer; NPC, neural progenitor cell; OPC, oligocendrocyte progenitor cell; PBMC, peripheral blood mononuclear cell; PML, progressive multifocal leukoencephalopathy; PPMS, primary progressive MS; RA, rheumatoid arthritis; RNS, reactive nitrogen species; ROS, reactive oxygen species; RRMS, remitting-relapsing MS; SASP, senescence-associated secretory phenotype; SLE, systemic lupus erythematosus; SPMS, secondary progressive MS; TCR, T cell receptor; Th, T helper cell; TREC, T cell receptor excision circles; Treg, regulatory T cell.

cancer (due to an imbalance between the function of regulatory cells and cytotoxic $CD8^+$ T cells) and auto-immune diseases (due to reduced clearance of apoptotic cells and reduced antibody diversity, with however an increased susceptibility to molecular mimicry) (27–30). These risks might be counterbalanced by the subject's intrinsic (e.g., genetic polymorphisms, epigenetics) and extrinsic factors (e.g., the individual's history of past immune reactions, referred to as immunobiography, as well as environmental factors) (11, 15).

T Cells

With aging, the pool of naïve T cells is reduced due to thymic involution and reduced bone marrow proliferative capacity. Both the thymus and the bone marrow lose their epithelial/stromal cell frame, which is replaced by adipocytes, resulting in a reduction in HSC proliferation (31, 32). Moreover, a general shift of HSCs from the lymphoid to the myeloid lineage is observed. Thymic T cell output, measurable by T cell receptor (TCR) excision circles (TRECs), is reduced with age. TRECs are stable extrachromosomal DNA byproducts resulting from thymic TCR rearrangements. TRECs are not replicated and are therefore diluted with cell division (33).

Homeostatic proliferation, driven by dendritic and B cells upon exposure to IL7 and IL15, occurs initially to compensate for the reduced peripheral input of naïve T cells, but results in the clonal expansion of memory T cells and a depleted TCR repertoire (34–37). The proportion of T helper (Th) cells decreases due to defective antigen presentation and an impaired TCR response, resulting in a reduction of TCR-mediated proliferation (38). The reduced expression of CD40 ligand (CD40L) on $CD4^+$ T cells impairs their binding to B cells and thus their ability to function as T helper cells (39).

With aging, a shift from Th1 to Th2 cells is observed, due to decreased IL2 production, although this is disputed (40, 41), while the percentage of Th17 cells is increased in subjects aged over 65 as compared to younger subjects (42). Moreover, memory T cells are resistant to apoptosis, hence reenforcing their numerical increase (43).

Overall, during senescence, the number of $CD4^+$ T cells decreases and $CD8^+$ T cells increases resulting in an inverted $CD4^+/CD8^+$ ratio (<1) (10). Antigen-experienced T cells proliferate and differentiate into terminally differentiated memory cells with shortened telomeres that eventually lose CD28 expression, a costimulatory signal involved in T cell activation and survival (44). This loss, mainly observed in memory $CD8^+$ T cells, has been linked to aging and immunosenescence, and is partly enhanced by chronic antigenic stimulation, especially by CMV, with a ten-fold factor for $CD4^+$ and 2-fold for $CD8^+$ T cells (45, 46). These $CD28^-$ cells express the natural killer (NK) receptor NKG2D which provides an antigen-independent activation signal (along with the NK adaptor molecule DAP12), bypassing the missing costimulatory signal CD28, and enhancing their autoreactivity (47, 48). Moreover, these cells express cytokines [interferon (IFN)g, TNFa] and cytotoxic molecules (granzyme A/B, perforin) upon expression of the eomesodermin factor, and are resistant to apoptosis [by expressing B cell lymphoma 2 (BCL2) and Fas-associated death domain-like IL-1-converting enzyme inhibitory protein (FLIP)] (49–51). Finally, they express chemokine receptors [e.g., C-X3-C Motif Chemokine Receptor 1 (CX3CR1)], which might favor their migration to inflammation sites (51, 52).

In summary, immunosenescence in T cells is characterized by a physiologically reduced pool of naïve T cells and an increase in memory, particularly $CD8^+$, T cells, that have lost CD28 and express NKG2D, the first increasing T cell self-reactivity in secondary lymphoid organs, the second reducing their threshold for antigen-specific activation hence enabling an antigen-independent activation. The autoreactivity of senescent T cells is enhanced by the clonal expansion of memory T cells and the reduced TCR repertoire. These changes can also partly explain the reduced immune defenses against new pathogens observed during aging, as senescent cells are considered functionally deficient, contrary to exhausted T cells, which are considered dormant and can still respond to a previously encountered antigen (15).

B Cells

B cell numbers, phenotypes and functions change with age [reviewed by (53, 54)]. Reduced B cell output is attributed to global changes in hematopoiesis, as described above. Moreover, peripheral B cell survival factor levels, such as B cell activating factor (BAFF) and A proliferation-inducing ligand (APRIL) are reduced in the elderly (55). In addition, stromal cell-derived IL7 production is reduced, whereas the increased pro-inflammatory cytokine levels [TNFa, IL1b, and transforming growth factor (TGF)b] withhold the B progenitor cells from the IL7-rich niches, hence impairing B lymphopoiesis and reducing the pro-B cell immunoglobulin (Ig) heavy chain V-DJ rearrangement and thus the pre-B cell receptor (BCR) repertoire (56–59). As a consequence, absolute and relative numbers of peripheral $CD19^+$ B cells are reduced, while the proportions of B subsets remain stable with age in humans (53).

With aging, naïve mature (IgD^+CD27^-) B cells decrease while exhausted double negative memory (IgD^-CD27^-) B cells increase. IgM unswitched (IgD^+CD27^+) and switched (IgD^-CD27^+) memory B cells remain generally stable (53, 54). The immature transitional immunoregulatory $CD24^{high}CD38^{high}$ B cell subset decreases with age, so does its IL10 production (60).

The humoral immune response is altered during senescence, since antibodies are reduced not in quantity but in their diversity and affinity and show cross-reactivity to self- and foreign antigens. This is due to a decrease in antibody class switch and affinity maturation in clonally expanding B cells, related to the downregulation of the E47 transcription factor and activation-induced deaminase (61, 62). This alters the ability to mount a rapid secondary antibody response. Furthermore, a progressive decline in germinal center formation during aging decreases somatic hypermutation, *i.a.* in IgD^-CD27^+ B cells and even more in double negative B cells (63). Moreover, immunosenescent B cells lack the support of the Th cells, since Th cells are reduced in number, express less CD40L, and are less exposed to antigen presentation by antigen presenting cells (APCs), due to a reduced expression of the major

histocompatibility complex of class II (MHC-II) on the latter (39, 64).

Interestingly, double negative memory B cells express chemokine receptors, C-X-C Motif Chemokine Receptor 3 (CXCR3), although reduced with age, C-C Motif Chemokine Receptor (CCR)6 and CCR7, and are thus prone to migrate to the inflammation sites (65). Moreover, these cells are pre-activated and can produce pro-inflammatory cytokines, and granzyme (66, 67). They undergo an antigen-driven BCR hypermutation.

Finally, low but steadily expanding $CD11b^{+}CD11c^{+}CD21^{-}$ age-associated B cells (ABCs) have been identified in the elderly, in response to antigenic stimulation, and linked to autoreactivity. This functionally exhausted memory subset is driven by the T-box transcription factor (TBET) and is activated synergically upon stimulation of the BCR and Toll-like receptors (TLR)7 and 9. ABCs produce pro-inflammatory cytokines (e.g., TNFa), inhibit B lymphopoiesis and favor Th17 polarization (68–70). They possibly derive from follicular B cells and exhibit downregulation of Epstein Barr Virus (EBV) receptor CD21 due to chronic EBV stimulation.

In summary, the changes in B cell phenotypes, and recirculation, along with their altered humoral response contribute to immunosenescence and can explain the reduced response to vaccination and increased susceptibility to infections, while the clonal expansion of B cells cross-reactive to self-antigens can favor autoimmunity.

Immunosuppressive/Regulatory Cells

Natural, thymic-derived $CD4^{+}CD25^{+}FOXP3^{+}$ regulatory T cells [(n)Tregs], mainly with an effector memory phenotype ($CD45RO^{+}/CD45RA^{-}$), increase with age in human in relative and absolute numbers, so does the expression of their transcription factor, forkhead box P3 (FOXP3), possibly due to their better survival in the periphery, since they reduce the expression of the pro-apoptic BCL2 interacting mediator of cell death (BIM) (71–74). Functionally, $CD4^{+}$ Tregs of aged humans and mice can suppress $CD4^{+}$ and $CD8^{+}$ T cell proliferation and IFNg production, but in aged mice they could not suppress IL17 production (75, 76).

Likewise, natural $CD8^{+}FOXP3^{+}$ nTregs increase with age, while their peripheral inducible capacity is reduced (77, 78). Functionally $CD8^{+}$ nTregs retain the same suppressive ability independently of aging. Interestingly, a $CD8^{+}CD28^{-}FOXP3^{+}$ cell subset has been described, in agreement with the overall increase of $CD8^{+}CD28^{-}$ T cells (79). Finally regulatory B cells and myeloid-derived suppressor cells also appear increased with age but have been less studied (80).

In summary, Tregs participate to the immunosenescent process by their increased number and their safeguarded suppressive activity, except against Th17 cells [reviewed by (79, 81)]. This correlates with increased cancer incidence, since Tregs suppress the $CD8^{+}$ T cell anti-tumor response, and with an increased risk of infection and viral reactivation, since they suppress the anti-pathogen response (72, 82, 83). They have also been linked to neurodegeneration due to their differential interaction with microglia both in the presence and absence of effector T cells (84).

Innate Immunity

Although less affected by immunosenescence, partly because HSCs are redirected toward the myeloid lineage, innate immunity still displays mainly functional changes [reviewed by (85)].

With aging, dendritic cells show less migration abilities, less responsiveness to TLR stimulation, reduced pathogen processing (phagocytosis, endocytosis) and antigen presentation. This is attributed to mitochondrial dysfunction, resulting in the production of reactive oxygen species (ROS) (86, 87). These alterations affect T cell stimulation and consequently the $CD8^{+}$ T cell cytotoxic response. Type I (IFN-I, i.e., IFNa/b) and III (IFN-lambda) IFN production is decreased, but they still produce IL6 and TNFa (87, 88).

Several important functions of neutrophils are reduced with aging: chemotaxis, phagocytosis, production of ROS and neutrophil extracellular traps (NET). Opsonization of antibody-bound pathogens is dwindled (89, 90).

Monocytes shift from the classical ($CD14^{++}CD16^{-}$) to the pro-inflammatory non-classical ($CD14^{+}CD16^{++}$) phenotype, however with some discrepancies on their expression of Human Leukocyte Antigen-DR isotype (HLA-DR) and CX3CR1 (91–93).

Macrophages also produce less ROS, IL6, and TNFa. They display impaired phagocytosis resulting in reduced antiviral response and impaired auto-/mitophagy, resulting in the accumulation of altered organelles and molecules (25, 94). Moreover, they express less TLRs and MHC-II on their surfaces, thus impairing their ability to present antigens to $CD4^{+}$ T cells (95, 96).

The slight net increase in the total number of NK cells is in fact due to a decrease of the immunoregulatory $CD56^{bright}$ and an increase of the cytotoxic $CD56^{dim}$ NK cell subsets. These show however impaired degranulation and thus decreased cytotoxic abilities on a per cell basis. IL2/12-mediated secretion of immunomodulatory cytokines (e.g., IFNg) and chemokines is reduced, while production of IL1/4/6/8 and TNFa is increased (97, 98). Furthermore, the central maturation of NK cells is incomplete (99).

Epigenetics and Telomeres

Insight into the function of microRNAs (miRNAs) has rapidly grown over the past two decades. miRNAs are small non-coding RNAs regulating gene expression post-transcriptionally by binding to their target messenger RNA (mRNA) and mostly inhibiting its translation (100). Overall, miRNA transcription decreases with age [e.g., miR-17/92a/181a in peripheral blood mononuclear cells (PBMCs)] (101, 102), and they have been linked to several mechanisms underlying cellular senescence [reviewed by (103)]. Oxidative stress can affect positively or negatively miRNA expression (104–107). On the opposite, the downregulation of miR-146a enhances NADPH oxidase (NOX) as it targets its subunit *NOX4* (108). The downregulation of the miR-17-92 cluster and the upregulation of miR-210 enhance ROS production (109, 110). Furthermore, miR-210 induces senescence-associated heterochromatin loci and double-strand DNA breaks and is involved in mitochondrial dysfunction by targeting a subunit of the electron transport chain (110, 111).

miR-34a and -101 inhibit autophagy (112, 113). By targeting the pro-proliferative cyclin A2, an antagonist of p21, miR-29a and -124, both induced by p53, enhance p21 expression, and thus senescence by cell cycle arrest (114). miR-20a is also an indirect p53-senescence inducer (115). Furthermore, the miR-17-92 cluster and miR-106b family target *p21*, while p53 inhibits the miR-17-92 cluster (107, 116–119). Finally, miR-9/96/145 were upregulated concomitantly to the downregulation of insulin growth factor 1 receptor (a miR-96/182-target) and forehead box protein O1 (FOXO1, a miR-145/132-target) in the PBMCs of elderly subjects, but miR-132 and -182 were not differentially expressed in this study (120).

Telomeres [reviewed by (121)] are repetitive hexameric sequences (TTAGGG) at the chromosome end of 10–15 kb at birth that shorten by 40–200 bp with each cell division, although length of shortening per mitosis might vary as it is higher in memory than in naïve cells. Critically short telomeres induce a signal for p53-dependent cell cycle arrest. Telomerase is a ribonucleoprotein complex comprising a catalytic subunit, telomerase reverse transcriptase (TERT), which can elongate the hexameric sequences. Telomere length depends on the balance between telomere shortening and telomerase activity, but overall decreases with age. Telomerase activity is increased in stem cells, but also in lymphocytes, where it is the highest in the germinal center (122, 123). However, this activity in the latter is not enough to slow down telomere shortening. Oxidative stress and an increased replication rate upon repetitive stimulation during inflammation, progressively reduce telomerase activity, paralleling the loss of CD28, and hastens telomere attrition and thus cellular senescence (121, 124, 125). Remarkably, centenarians have longer telomeres with lower levels of basal inflammation (14). Interestingly, miRNAs can induce telomere dysfunction and cellular senescence, as miR-138 and -512-5p inhibit *TERT*. miR-155 targets telomeric repeat-binding factor *(TRF)1*, miR-23a targets *TRF2*, which both ensure telomere maintenance (126–129).

Several miRNAs are considered as major immuno-microRNAs, playing a role in immune cell homeostasis and senescence, but also in inflammatory responses and inflammaging [reviewed by (130, 131)]. Age-dependent changes in miRNAs diverge between naïve, central and effector memory $CD8^+$ T cells, but miR-181a is commonly downregulated in the aged cells of all 3 subsets. The most changes are uncovered in the naïve T cell subset, where they are correlated with the decline of FOXO1 signaling, evidenced by the downregulation of IL7 receptor and *CCR7*, and the alteration of TNFa and NF-kB signaling (132). The SASP in senescent cells induces the delayed expression of miR-146a/b to target IL1 receptor associated kinase (*IRAK*)*1* and to compensate downstream NF-kB-dependent inflammation mediated by IL6/IL8 (133). miR-223 downregulates the NF-kB pathway and the inflammasome NLRP3 (134). Contrarily, NF-kB induces miR-155, which inhibits suppressor of cytokine signaling *(SOCS)1*, allowing T effector expansion and T memory formation and maintenance (135). miR-17/19b/20a/106a were downregulated in $CD28^-$ vs. $CD28^+$ and in $CD28^+$ T cells of old vs. young donors alongside the upregulation of *p21* (109). The miR-17-92 cluster and miR-21 support the differentiation into T effector cells (136, 137). On the contrary, the T effector response upon viral infection is delayed in miR-155- or miR-181a-deficient ($CD8^+$) T cells, and cells differentiate to central rather than effector memory cells (138, 139). Moreover, with age, the decrease of Yin-Yang 1 and T cell factor 1 results in the downregulation of miR-181a, which induces dual specific phosphatase *(DUSP)6* expression. The latter impairs extracellular signal-regulated kinase (ERK)-dependent TCR sensitivity (140). Furthermore, miRNAs can impair B cell differentiation in the elderly. miR-155, that targets activation-induced deaminase, and miR-16, that targets *E47*, are increased in memory B cells and even more in double negative B cells (141, 142).

Epigenetics translate the effect of the environment on gene expression [reviewed by (143)]. While methylation, catalyzed by DNA methyltransferases (DNMT), can vary at a cell-base level, the global methylation rate is reduced with age, possibly by passive demethylation and reduced activity of DNMT1 (144). Hypomethylation allows gene expression. Naïve $CD4^+$ T cells display age-associated hypomethylation sites in immune-related pathways [TCR signaling, Fc gamma receptor (FCgR)-mediated phagocytosis, mammalian target of rapamycin (mTOR) and insulin signaling, antigen processing and presentation], while hypermethylation was observed in cell proliferation pathways [Wnt and mitogen-activated protein kinase (MAPK) signaling] (145, 146). Interestingly, centenarians display a slower reduction of DNA methylation level (147). Sirtuins (SIRT) encode NAD^+-dependent histone deacetylases (HDAC) and maintain the genome's integrity during cellular stress. Downregulation of *SIRT1* and *SIRT3* in PBMCs of healthy elderly subjects was accompanied by the upregulation of miR-9, which targets *SIRT1*, and miR-34a (148). Oxidative stress induces miR-195, that targets *SIRT1*, which is associated with reduced telomerase activity (149).

In summary, telomeres shorten with age due to cell replication and oxidative stress, despite sustained telomerase activity. Epigenetically, global hypomethylation occurs with aging. Interestingly, miRNAs are involved in cellular senescence through different mechanisms [oxidative stress, mitochondrial dysfunction, cell cycle arrest (p53 pathway), telomere attrition, and inflammation].

IMMUNOSENESCENCE IN AUTO-IMMUNE DISEASES

Immunosenescence is associated with an increase in the incidence of several AIDs (150). Some diseases show a bimodal age of onset (e.g., RRMS vs. PPMS, MOG-antibody diseases), others almost exclusively occur in the elderly (e.g., polymyalgia rheumatica, giant cell arteritis) (30, 151–153). A German study showed an age-decreasing female incidence of SLE peaking at the age of 20–25 and to a lesser extent at menopausal age, and an age-increasing male incidence peaking at the age of 65–70 (154). RA-incidence and prevalence increase with age to peak at age 50–54 and 60–64, respectively (155). Serum autoantibody titers are generally higher in elderly subjects, even without overt AID. Moreover, the binding of circulating antibodies to random

peptides, especially with a di-serine motif, increases with age (150, 156).

Interestingly, AIDs show inflammaging and features of immunosenescence at an earlier age. Age-associated defects at the cellular level, classified under the nine common denominators of aging (2), and the resulting impaired immune function create an unstable state, that may predispose for tolerance failure and occurrence of autoimmunity (152, 157). Herein, we will focus on the effect of stem cell exhaustion, altered intercellular communication (e.g., by inflammaging), proteostasis (i.e., the maintenance of a functional cellular protein pool) loss, telomere attrition, genomic instability, mitochondrial dysfunction, and epigenetic alterations in MS, SLE, and RA.

Lymphopenia-induced homeostatic proliferation leads to clonal expansion and TCR repertoire contraction over time. Furthermore, priming by cytokines produced during inflammaging, can transiently reduce the TCR stimulation threshold (by ERK phosphorylation), consequently interfering with tolerance maintenance and promoting autoreactive T cells [reviewed by (152)]. TNFa engages cellular senescence by inducing interferon response genes, cytokine secretion, and ROS production (158). Moreover, the Th17/Treg imbalance contributes to trigger autoimmune diseases (42).

Three cell types are unique to immunosenescence, i.e., $CD28^-$ T cells, linked to cytotoxicity, double negative B cells and ABCs, linked to autoantibody production, and might play a role in autoimmunity (48, 66, 68). The $CD4^+CD28^-$ cell population is enlarged in subjects with autoimmune diseases compared to age-matched controls, with the highest percentage in RA, followed by RRMS and SPMS and finally SLE, and is positively correlated with age and CMV seropositivity. These cells are enriched in granzyme A and B, and perforin and their TCR repertoire is contracted as compared to $CD28^+$ T cells. The latter appears even stronger in MS/RA than in healthy controls (159).

Cells recycle long-lived proteins, damaged organelles, and aggregates by autophagy via the lysosomes, for the synthesis of new proteins or for energy production, thus ensuring cellular homeostasis, especially under nutrient-/energy-poor conditions (160, 161). Autophagy declines with age, as seen by the downregulation of autophagy-related protein (ATG)5, ATG7 in the human aged brain (162, 163). Interestingly, autophagy and inflammation can reciprocally induce and suppress each other. Autophagy is induced by TLRs but inhibited by Th2 cytokines. Conversely, it blocks the inflammasome, and thus the IL1b response. It prevents ROS production by degrading dysfunctional mitochondria, but it also promotes the survival and differentiation of immune cells [reviewed by (164)].

In AIDs, metabolic reprogramming for energy production may fail leading to hyperreactive immune cells and an increase in oxidative stress. Oxidative stress and mitochondrial dysfunction contribute to (immuno-)senescence and inflammation through decreased redox capacity (glutathione depletion), activation/oxidation-induced cell apoptosis (with defective clearance and release of the cell content inducing TLR), mitochondrial DNA (mtDNA) damage, defective bioenergetics (ATP depletion) and production of neoantigens (165, 166). Moreover, oxidative stress, inflammation, and increased leukocyte renewal accelerate telomere shortening [reviewed by (166)].

Immunosenescence in Multiple Sclerosis

In MS, activated peripheral autoreactive $CD4^+$ T cells, migrate through a disrupted blood brain barrier (BBB) into the CNS. They are reactivated upon antigenic contact, and interact with other peripheral immune cells ($CD8^+$ T and B lymphocytes, monocytes, and macrophages). They activate microglial cells and astrocytes to induce demyelination, oligodendrocyte apoptosis and axonal damage (**Figure 1A**) (18). MS patients present at a younger age with some features of immunosenescence seen in aged healthy controls, suggesting that it is possibly involved in MS pathogenesis [reviewed by (19, 167)].

Innate Immunity

Circulating neutrophils in RRMS patients produce more inflammatory markers and NETosis, and are resistant to apoptosis. The serum/plasma levels of neutrophil-activating chemokines and neutrophil-derived enzymes [e.g., C-X-C motif ligand (CXCL)1, CXCL8, elastase] are positively correlated with new inflammatory lesions. Neutrophils are also found in the cerebrospinal fluid (CSF) at onset and early in relapse, but decrease with disease duration (168–170). Regarding monocytes, discrepancies exist due to study population and staining strategy differences. Some describe an increase in classical and non-classical monocytes in inactive RRMS as compared to progressive MS, while others state an increase in non-classical monocytes in a mixed MS population (171, 172). The beneficial or detrimental role of NK cells is still debated [reviewed by (173)]. The peripheral blood of PP/SPMS counts more $CD56^{dim}$ NK cells, while the $CD56^{bright}$ NK cells are expanded in the CSF of untreated RRMS patients due to their higher migratory capacity, which might counterbalance the CNS inflammation (174, 175). However, their immunoregulatory and cytolytic functions appear to be impaired (176, 177). Moreover, NK cells in the CNS could delay remyelination, as they suppress the reparative properties of neural stem cells in experimental autoimmune encephalitis (EAE) (178).

T Cells

The bone marrow cellularity is reduced and the *in vitro* proliferative capacity of mesenchymal stromal cells, supportive of hematopoiesis, declines with age, even more in PP/SPMS, while $CD34^+$ HSC numbers remain stable in MS but the frequency of colony forming cells is low (179, 180). Moreover, thymic involution is accelerated in MS, given that TREC levels are, at all ages lower than in age-matched healthy controls and progressively decrease with age (159). Hence, the $CD8^+$ naïve T cell pool is reduced, while data regarding the effect of age on naïve $CD4^+$ T cells in MS are more discrepant (181–183). Interestingly, the TCR repertoire is more diversified in MS (184). Moreover, Th17 cells are largely involved in MS and increased in the periphery (185, 186). The inverted $CD4^+/CD8^+$ ratio, the hallmark of the immune risk phenotype, mostly does not apply in MS (187–189). This ratio was however

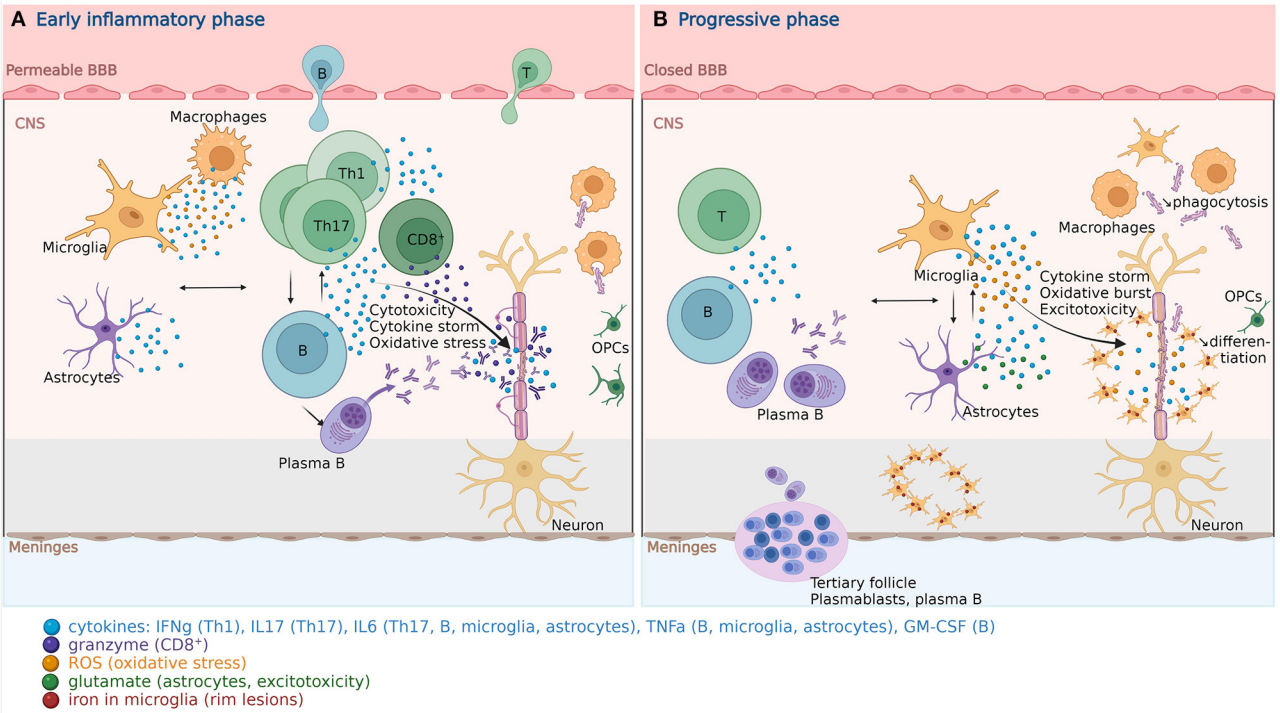

FIGURE 1 | Pathophysiology of multiple sclerosis. **(A)** In the early inflammatory phase of MS, peripheral adaptive immune cells infiltrate the CNS through a disrupted BBB. These activated cells interact with each other and the resident cells of the CNS. They secrete cytokines (e.g., IFNg by Th1, IL6/17 by Th17, GM-CSF, IL6, TNFa by B cells) and cytotoxic molecules (e.g., granzyme B by $CD8^+$ T cells). B cells can further evolve into autoantibody-producing plasma cells. As a consequence, T and B cells activate macrophages and microglia, which produce cytokines, nitric oxide, and ROS. This cytotoxic pro-inflammatory environment breaks down the myelin sheaths around axons and induces energy failure in the axon. Macrophages and microglia can still clear the myelin debris, allowing for the recruitment of OPCs that will partially remyelinate the lesion. **(B)** In the progressive phase of MS, T and B cell infiltrates are reduced. Remarkably, plasmablasts and plasma B cells form tertiary follicle-like structures in the meninges. The BBB is closed and the inflammation is sustained by innate CNS-resident cells, i.e., microglia and astrocytes. They produce cytokines (TNFa, IL6) and release ROS, causing myelin damage. Although microglia are primed into a pro-inflammatory phenotype, their phagocytic capacities are reduced. These features also characterize the senescence-associated phenotype of microglia and astrocytes. Myelin debris are improperly cleared, OPCs are less recruited and fail to differentiate. TNFa-mediated glutamate release from astrocytes results in excitotoxicity causing axonal damage. The ferrous iron released from the myelin, where it accumulates with age, is oxidized, which produces ROS, and incorporated by microglia, forming a phenotypical rim around the demyelinating lesions, both in the white and gray matter. These successive events are self-sustained and enhanced by senescent processes, resulting in a major oxidative burst, causing mitochondrial dysfunction, mitochondrial DNA damage, energy failure and axonal loss. BBB, blood brain barrier; B, B cell; CNS, central nervous system; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; MS, multiple sclerosis; OPC, oligodendrocyte progenitor cell; ROS, reactive oxygen species; T, T cell; Th, T helper cell; TNF, tumor necrosis factor. Created with BioRender.com.

decreased in the CSF of patients on natalizumab treatment (190, 191).

Both effector memory $CD4^+$ and $CD8^+$ T cells may enhance the chronic inflammatory responses to neuroantigens in MS and EAE (192, 193). Notably, inoculation with $CD4^+$ memory rather than effector T cells in EAE preferentially induced marked CNS inflammation (194, 195). Herein, memory $CD4^+$ T cells are increased in the blood and the CSF during active disease (196). Central and effector memory $CD8^+$ T cells are increased, independently of disease activity, in the blood, and the latter also in the CSF, and the CNS tissue (192, 196–198).

The cytotoxic $CD4^+CD28^-$ population is enriched with advancing age in RR/SP/PPMS, while it remains stable in healthy controls, and has been linked to disease severity in EAE and MS (159, 199–201). They are partly autoreactive to myelin basic protein (MBP) (199). Since these cells express CX3CR1, they might infiltrate the CNS where the CSF levels of its ligand fractalkine were found elevated in MS (52).

B Cells

B cells play a central role in MS development and progression [reviewed by (202)]. Antigen-driven clonally expanded B cells produce pro-inflammatory cytokines [TNF, lymphotoxin (LT)a, IL6, granulocyte-macrophage colony-stimulating factor (GM-CSF)] and chemokines through the NF-kB pathway. Memory B cells act as APCs, and hereby prompt the proliferation and activation of T and myeloid cells. B cells, stimulated by Th follicular cells, differentiate into immunoglobulin-producing plasmablasts and plasma cells that accumulate to eventually form tertiary follicle-like structures in the leptomeninges during disease progression and are notably involved in inducing subpial demyelination.

Transitional B cells ($CD24^{high}CD38^{high}$) are reduced in the blood and are functionally defective in RRMS (produce less IL10). They have been found in the CSF while they were absent in CSF samples of other inflammatory neurological diseases (203, 204). The proportions of peripheral naïve B cells decrease and memory B cells increase with age in controls (54), but remain stable in MS, except during relapse. Interestingly, the proportion of CSF class-switched memory B cells is increased in adult MS whereas the relative numbers of unswitched memory B cells are increased in pediatric MS (205). In B cells from MS patients, a preferential naïve-to-memory transition possibly occurs as the production of LTa and TNFa by memory $CD27^+$ B cells was high and comparable to that of healthy controls, whereas the production of IL10, normally expressed by naïve $CD27^-$ B cells was reduced (206).

Double negative (IgD^-CD27^-) B cells and ABCs ($CD11c^+CD21^-$ or $CD21^{low}$) are increased in a proportion of MS patients before the age of 60 years, whereas they are mainly found above 60 in healthy controls. This increase is positively correlated with age in healthy controls but not in MS patients and with $CD4^+CD28^-$ T cell numbers in all subjects (63, 67). Remarkably, double negative B cells and ABCs are also increased in the CSF of MS patients (67). Double negative B cells from MS patients have a higher activation potential than those from controls. They are involved in antigen presentation as well as costimulation, and can produce proinflammatory cytokines (TNFa, LTa), and granzyme B after stimulation (67).

Tregs

In RR/PPMS, Tregs ($CD4^+CD25^+CD127^-$) levels were stable as compared to controls, although resting Tregs ($CD45RA^+CD25^{dim}$) were reduced, while activated Tregs ($CD45RA^-CD25^{bright}$) were increased in active MS (207, 208). There are some discrepancies whether Treg numbers and expression of surface markers are different between MS and control subjects (207–209). Overall, it is considered that the suppressive activity of Tregs is reduced in (RR)MS, but it seems to improve in SPMS (210–213). Several miRNAs have been found to target the TGFb pathway limiting the differentiation of $CD4^+$ naïve T cells to Tregs. These miRNAs, however, did not affect their suppressive function (214).

Inflammatory Mediators/Inflammaging

CSF levels of TNFa, CXCL10 and IL8 increased with age in healthy controls, while IL10 level was the lowest in the middle age group (40-to-59-years-old). This inflection point of IL10 production possibly occurs 10–20 years earlier in MS, due to premature immunosenescence, which might correspond to disease onset. On the contrary, in MS, TNFa, CXCL10, IL8, and IL10 levels were higher than in controls, but only IL8 and CXCL10 increased with age. Moreover, there is a shift from Th1 to non-Th1 cytokine profiles in aging and MS, as the age-related increase of CXCL10 was relatively lower than for the other cytokines (41).

Circulating and CSF levels of a few markers are overall in favor of the presence of inflammaging in MS, although there are some discrepancies between studies. IL6 and TNFa are increased in the serum and the CSF in RRMS, mainly relapse, and SP/PPMS. IL6 correlates with disease duration, serum TNFa in PPMS correlates with disease progression. Serum IL10 levels were increased with remission, while CSF levels were high during relapse [reviewed by (215)].

Proteostasis/Autophagy

Autophagy is increased in active RRMS, evidenced by the upregulation of ATG5 in peripheral T cells and in encephalitogenic T cells on brain autopsy samples (216). It exhibits both detrimental and protective effects dependent on the cell type. It enhances neuroinflammation by supporting autoantigen presentation by DCs and the survival of autoreactive B and T cells. Conversely it protects neuronal integrity, oligodendrocyte survival and the fragile pro/anti-inflammatory balance in astrocytes and microglia [reviewed by (217)]. However, sustained autophagy due to unresolved damage might lead to its detrimental dysregulation, paradoxical inflammasome activation and apoptosis (162, 218).

Telomeres/Telomerase

Telomeres in whole blood DNA (thus mainly PBMCs) were shorter in all MS subtypes (219) as compared to controls, and their length was negatively correlated with age. Telomere shortening was associated with a higher relapse rate, disability, and brain atrophy (220). It was predictive of transition to SPMS (220, 221).

Oxidative Stress/Mitochondrial Dysfunction

Peripheral lymphocytes of MS patients exhibit an increased glucose demand with impaired oxidative phosphorylation, alongside mitochondrial dysfunction (marked by a reduced enzymatic activity and a decoupling of the respiratory chain) (222–224). Concurrently, oxidative stress can promote T cell activation and Th17 differentiation (225–227). Interestingly lymphocytic resistance to apoptosis might partly be due to an impaired mitochondria-mediated apoptotic deletion, as observed in $CD4^+CCR5^+$ T cells of PPMS (228).

Epigenetics

Contrary to aging, methylation appears to be globally increased in MS [reviewed by (229)], with different methylation profiles between MS phenotypes, higher in PPMS compared to RRMS (230), but slightly higher in RRMS compared to SPMS (231). Lymphocyte signaling, T cell activation and migration were common pathways to RRMS and SPMS methylation profiles, while myeloid cell function and neuronal and neurodegenerative genes and pathways were SPMS-specific (231). Thirteen N6-methyladenosine (m6) regulatory genes were overexpressed in the CSF of MS patients as compared to healthy controls, of which 9 were negatively correlated with age. Remarkably, non-supervision consensus clustering separated RRMS and progressive MS patients in 2 distinct clusters, with higher levels of the m6 regulatory genes and m6 RNA methylation in RRMS patients (232).

miRNAs are upregulated in the CSF of mainly relapsing MS patients and associated to inflammatory (NF-kB, FOXO, TNFa, TGFb), cell cycle and p53 signaling pathways (233).

miR-155-5p targets *SOCS1* and hence promotes Th17 and Treg differentiation, and microglia-mediated immune response through expression of IL6, TNFa and induced nitric oxide synthase (iNOS) (234, 235). It also disrupts the BBB while miR-146a-5p protects it by modulating leukocyte adhesion to endothelial cells (236, 237). Moreover, miR-146a-5p inhibits Th17 differentiation by repressing TNF receptor-associated factor *(TRAF)6* and *IRAK1*, transducers of NF-kB (238). Remarkably, miR-150 that targets *CMYB*, promotes terminally effector rather than precursor memory $CD8^+$ T cells and is also expressed in mature B cells (239, 240).

Relevance of Immunosenescence on MS Disease Features

Resident Cells of the Central Nervous System

MS-related inflammatory processes influenced by immunosenescence, potentially alter the function of CNS-resident cells by promoting senescence and a pro-inflammatory phenotype, which enhances the oxidative burden, resulting in alteration of mitochondrial function and DNA integrity. Moreover, cell cycle arrest and phenotypic changes in senescent cells might affect their functions and their regenerative capacity (241).

Oligodendrocytes

The adult brain encloses its remyelination potential into a pool of oligodendrocyte progenitor cells (OPCs). OPCs represent 5–10% of all CNS cells, can undergo asymmetric division and migrate to the site of demyelination to differentiate into mature oligodendrocytes thereby forming new myelin sheaths. This remyelination potential naturally decreases and slows down with age [reviewed by (241, 242)]. In addition, OPCs are improperly recruited to chronically demyelinated MS lesions and fail to differentiate with disease progression, due to intrinsic and extrinsic factors (243, 244).

Intrinsic factors include age-related decline of histone deacetylation and methylation in OPCs and oligodendrocytes (by reduced HDAC class I expression), enhancing the heterochronic expression of transcriptional inhibitors [e.g., inhibitor of DNA-binding (Id)4] as well as global hypomethylation by downregulation of *Dnmt1* in OPCs of aged mice (245–247). Likewise, DNA methylation of ID2/ID4 allows OPC differentiation, but their methylation levels were lower in MS lesions on human brain samples than in controls (248). Extrinsic factors from the OPC environment can also affect their differentiation. Unlike induced pluripotent stem cell (iPS)-derived neural progenitor cells (NPCs) from age-matched healthy controls, NPCs from PPMS patients expressed senescence markers ($p16^{INK4}$, p53, increased senescence-associated beta-galactosidase activity), and failed to induce OPC differentiation. This was reversed by treating the NPCs (and not the OPCs) with rapamycin or a blocking antibody against high-mobility group box (HMGB)1, a mediator of neuroinflammation in the SASP of NPCs (249, 250).

Moreover, immune reactive OPCs can contribute to neuroinflammation and to their own functional impairment in demyelinating conditions, as they express IL1b, MMP9, MHC-I/II, and immunoproteasome genes, facilitating the early disruption of the BBB, the recruitment of activated immune and glial cells and their production of cytokines (e.g., IL6 by astrocytes) (242, 251–254). They are also involved in neuronal cytotoxicity, by enhancing glutamatergic transmission through IL1b or dysregulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors through IFNg, directly or indirectly by inducing lymphocytic cytokines (255–257). Inversely, the SASP of the surrounding cells can interfere with OPC differentiation (258).

Microglia

Microglia, maintained in a quiescent state by TGFb (259) and inhibitory ligand-receptor interactions with neurons, astrocytes, and oligodendrocytes, scan their environment through their ramifications, for danger signal and can sense extracellular ATP/UDP changes mirroring neuronal or astroglial injury/activity. Activated microglia will transiently change into pro-inflammatory subsets, particularly during myelin clearance, which sustains inflammation and hinders remyelination, while regulatory subsets support neuroprotection. However, their physiological age-related functional changes decrease their reparative ability toward CNS damage [reviewed by (260)].

Since microglia have a relatively long lifespan and a slow turnover rate, they are more prone to accumulate DNA damage and experience changes during aging [reviewed by (261)]. The motility and ramifications of microglia are reduced, and their sensome gene expression profile changes with age, delaying their recruitment to site and reducing their ability to sense their surroundings. Moreover, aged microglia are chronically activated and exhibit an elevated immunoreactivity and an exaggerated pro-inflammatory response, the so-called microglial priming. TLRs and advanced glycan-end products are upregulated while immune-suppressive factors (CD200R, CX3CR1) are downregulated, enhancing the expression of MHC-II, pro-inflammatory cytokines (IL1, IL6, TNFa), and the production of ROS/reactive nitrogen species (RNS) (by overexpressing NOS and NADPH oxidase) (262–265). Conversely, the age-related increase in TGFb levels, with senescence promoting roles [reviewed by (266)], induces changes in aging microglia that interfere with their ability to acquire a regulatory phenotype and to promote OPC differentiation (267). Moreover, activated microglia initiate a TNFa-mediated synaptic degeneration, and reciprocally influence astrocytes through TNFa and ATP to prompt the astroglial release of glutamate (257, 268).

With age, the phagocytic activity of microglia declines, impairing the clearance of myelin debris and delaying remyelination [reviewed by (269)]. Furthermore, as lysosomal degradation and cholesterol efflux are defective, lipofuscin granules (insoluble aggregates of myelin) accumulate, which increase inflammasome signaling and protein expression (270, 271).

Although microglia and macrophages are phenotypically related and complement one another in MS pathogenesis, their age-related changes partially differ. Aging macrophages are deficient in phagocytosis and chemotaxis, as microglia. Contrarily to microglia, they lose their pro-inflammatory and

regulatory functionality (i.e., reduced activation of NF-kB, downregulation of TLR4, TNFa, IL6) [reviewed by (269)]. Interestingly, transferring young macrophages into an aging demyelinating brain enhanced remyelination (272).

Astrocytes

Astrocytes are part of the CNS innate immune system and participate to demyelination by impairing the BBB, by controlling the passage of immune cells through the BBB (cellular adhesion molecules), by attracting peripheral immune cells and resident CNS cells to the lesion site (chemokines), by guiding T cell phenotypes, by inducing B cells (BAFF), by modulating microglial recruitment and function, and by acting as APCs. Although astrocytes can prevent excitotoxicity by glutamate uptake, they can worsen it by secreting several cytotoxic factors (ROS, RNS, glutamate, ATP) in response to IFNg and IL1b stimulation. Furthermore, TNFa downregulates glutamate receptors in astrocytes, thus elevating the extracellular levels of glutamate, which is directly toxic to oligodendrocytes, axons and neurons. Astrocytes further secrete fibroblast growth factor 2 (FGF2) and produce glycosaminoglycan hyaluronan, which promote OPC proliferation instead of their differentiation, and produce chondroitin sulfate proteoglycans, ephrins, and myelin-associated inhibitors, which inhibit axonal growth. The glial scars formed by reactive astrogliosis try to contain the demyelination by surrounding the damaged area, but their rigidity hinders remyelination and axonal regeneration [reviewed by (273)].

In aging astrocytes, the overexpression of the intermediate filaments, glial fibrillary acidic protein (GFAP) and vimentin, parallels increased $p16^{INK4}$ expression and cell cycle arrest. Moreover, TGFb1 and HMGB1 induce pro-inflammatory cytokines (IL6, TNFa, IL1b, prostaglandins) and chemokines constituting the SASP of aging astrocytes (274, 275). Interestingly, EAE improves by blocking HMGB1 in the CNS (276). Furthermore, during EAE, oxidative stress sustains excitotoxicity (273, 277).

Inflammatory Activity vs. Progression in Relationship to Aging

While 80–85% of patients present with RRMS at a younger age, the relapse rate is reduced with aging. Moreover, the post-relapse recovery potential decreases with age. The decline in white matter integrity and neuro-axonal reserve might precipitate the onset of progression, and increase the risk of accumulating disability (278–281). It is now established that subclinical neurodegeneration starts long before clinical progression becomes more evident, explaining the occurrence of progression independent of relapse activity (PIRA) in earlier phases of the disease (282). Therefore, according to natural history studies, up to 50% of RRMS patients might transition to SPMS, 15–20% present with disability progression from onset (PPMS) (18). Remarkably, both PPMS and SPMS onset occurs on average around the age of 45 years. Transition to SPMS happens independently of the duration of the prior relapsing course (283). Aging and underlying senescence might therefore, at least partially, be involved in the evolution and pathogenesis of the disease.

The CNS inflammatory infiltration and acute axonal injury are negatively correlated with age, while in inactive progressive MS, the CNS inflammation declines to the same level as in healthy controls (284). While RRMS is characterized by a disrupted BBB allowing the invasion of the CNS by peripheral immune cells, progressive MS is characterized by a compartmentalized CNS inflammation, behind a closed BBB [reviewed by (285, 286)]. Follicle-like structures, enriched in B and plasma cells, form in the meninges and these cells have a higher relative contribution within the infiltrates (287). Perivascular and parenchymal T/B cell infiltrates are limited. New active lesions are infrequent. Slowly expanding white matter lesions, also called smoldering lesions, with low-grade myelin destruction and axonal degeneration, are formed by a moderate lymphocytic infiltration and a dense network of reactive astrogliosis in their center (288, 289), surrounded by activated microglia and macrophages forming a narrow rim (290). Cortical lesions are frequent and are also mainly caused by activated microglia, resulting in synaptic and neuronal loss (285, 286). In the normal appearing white matter, the proinflammatory state induces microglial and astrocytic activation resulting in diffuse axonal injury (291).

During the progressive phase of the disease, the oxidative burst by activated microglia is prominent (285). Iron accumulates with age in the brain and is stored with ferritin in oligodendrocytes. The oligodendrocytes, harmed by inflammation/oxidation, release ferrous iron. Ferrous iron (Fe^{2+}) reacts with H_2O_2 to form ferric iron (Fe^{3+}) and a hydroxyl radical, which increases the oxidative stress (285). Ferric iron is incorporated by microglia and macrophages at the active lesion margins, forming the magnetic rim lesions detectable by MRI in about 50% of the cases (290). This iron uptake causes dystrophy of macrophages and microglia, leading to the secondary release of iron and fueling the oxidative stress. Although autophagy is increased in progressive MS in an attempt to ensure cellular homeostasis, it is not enough to compensate the mechanisms at play in the periplaque environment causing cellular senescence (216, 285). Moreover, the oxidative stress results in and is subsequently amplified by mtDNA damage and mitochondrial dysfunction of the respiratory chain complexes. Furthermore, synaptopathy, which happens also in normal aging brain, is caused by reduced neurotrophic factors and excitotoxicity resulting from a glutamatergic/gamma-aminobutyric acid (GABA)-ergic imbalance, as well as by pro-inflammatory cytokines (IL1b, IL6, TNF) of activated microglia, astroglia, and infiltrating lymphocytes (257, 292, 293). IL1b can also alter synaptic plasticity. Both are exacerbated by neuroinflammation and accelerated with age during MS [reviewed by (293)]. These features contribute to neurodegeneration and translate at the macroscopic level into accelerated brain atrophy, which can be viewed as premature aging of the MS brain, at a rate of 0.7–1% per year, compared to 0.1–0.3% per year in healthy subjects (285, 294, 295).

Exosome-associated miR-15b-5p/23a-3p/30b-5p/223-3p/342-3p/374a-5p and miR-432-5p/433-3p/485-5p are, respectively, up- and downregulated in RRMS vs. PP/SPMS (296). Interestingly, miR-15b-5p and -23a-3p are predicted to target *FGF2*, a promoter

of OPC migration present in active and in the periphery of chronic lesions and elevated in the CSF of RR/SPMS (297–299). miR-342-3p is required for NF-kB induction in TNFa-activated microglia (300). miRNAs dysregulated in cortical lesions as compared to myelinated gray matter, are involved in axonal guidance, TGFb, and FOXO signaling. Furthermore, miR-20a/25/29c/149* are associated to gray matter atrophy (301).

In summary (**Table 1**, **Figures 1B**, **2**), with disease progression, the involvement of the peripheral immune system becomes secondary, while increasing oxidative stress, sustained by the pro-inflammatory phenotype of glial cells, is the major mechanism causing mitochondrial dysfunction in all CNS-resident cells, inducing their complete functional decline (impaired clearance of myelin debris, impaired remyelination, energy failure, loss of neurotrophic support, release of neurotoxic factors), resulting in irreversible neurodegeneration.

Efficacy and Safety of Disease Modifying Therapies in Aging MS Patients

Disease modifying therapies (DMTs) are efficient to reduce clinical relapses and radiological disease activity in active MS. However, due to the predominant CNS-restricted inflammation concurrent to neurodegeneration, treatments for progressive MS remain scarce, possibly more effective to patients with superimposed active inflammation [reviewed by (302)]. Moreover, since clinical trials classically exclude patients over the age of 55 years, the safety and efficacy of DMTs in older MS patients is still debated, while these patients represent a growing proportion of the MS population (17, 302). In patients younger than 40.5 years, high-efficacy drugs (ocrelizumab, mitoxantrone, alemtuzumab, and natalizumab) initiated without delay, were more powerful than lower-efficacy drugs (fingolimod, dimethyl fumarate, interferon-beta, teriflunomide, and glatiramer acetate), but may already lose their benefits on disability progression after that age. However, this model could not distinguish benign from active MS courses. The same meta-analysis by Weideman et al. found DMT efficacy to be negatively correlated with age and predicted no efficacy of DMTs after the age of 53 years (303). Moreover, the intrinsic effects of DMTs on immune cells in addition to the age-related changes in the immune system might become deleterious for remyelination and immunosurveillance with age, since DMTs deplete, sequestrate or functionally impair lymphocytes (191, 304–307). Discontinuing DMTs in the elderly might be reasonable for these reasons, however, studies are sparse. Herein, stable patients discontinuing DMTs experienced the same time to relapse as patients still on DMTs but a shorter time to disability progression. The latter was also correlated with age. However, this study included MS patients from 18 years and older, and did not focus specifically on the elderly MS patients (308). For patients older than 45 years, a 4-year relapse-free disease course under DMTs was predictive of absence of relapse following DMT discontinuation while longer disease duration and higher EDSS were predictive of disability progression (309).

Since DMTs directly act on the immune system, there has always been a major concern about risk for cancer and infections (191, 310). While some found initially a slightly increased risk for cancer (e.g., urogenital, breast, CNS cancers, lymphomas, melanomas) (311, 312), the overall risk considering all current DMTs is not increased [reviewed by (313)], although a higher incidence of neoplasm with depletive DMTs (alemtuzumab, cladribine, ocrelizumab) was found by a meta-regression analysis, especially after the age of 45 years (314). Switching from DMTs, especially more than twice, was also a risk factor for cancer (311). Furthermore, awareness is raised for the possible link between natalizumab, fingolimod, cladribine, or alemtuzumab and several types of immune malignancies, melanomas, carcinomas *etc.* [reviewed by (313)].

Natalizumab, dimethyl fumarate, and fingolimod increase the risk of progressive multifocal leukoencephalopathy (PML) caused by JC-polyomavirus. The risk related to natalizumab remains for several months after switching to another DMT, which explains carry-over cases as seen with ocrelizumab, fingolimod, or teriflunomide. Age, later age at DMT initiation (>50 years), prior immunosuppressive treatment, and lymphopenia, particularly inside the CNS, are important risk factors for PML, although cases with normal blood lymphocyte count have been reported (310, 315–317).

Other opportunistic infections increasing with age can be cryptococcal meningitis and herpes encephalitis (fingolimod, natalizumab) (318, 319), mucocutaneous herpes infection [sphingosine-1-phosphate receptor modulators, natalizumab, alemtuzumab], and varicella-zoster reactivation [sphingosine-1-phosphate receptor modulators, dimethyl fumarate (320), natalizumab, cladribine, alemtuzumab, ocrelizumab], human papilloma virus (fingolimod), and Listeria meningitis (alemtuzumab) [reviewed by (310, 321)].

Most DMTs act through different mechanisms on the subsets of the adaptive immune system that might undergo immunosenescence in parallel. For example, DMTs have been found to differentially affect the thymic and bone marrow output after treatment initiation but also in an age-related fashion. Both are, respectively, measured through TRECs, which decrease with age in healthy control, and K-deleting recombination excision circles (KRECs), which remain stable. While TREC levels did not change between DMT-naïve and DMT-discontinued patients, KREC levels were significantly enhanced in the latter. Interestingly, fingolimod that sequestrates lymphocytes in lymph nodes, showed a reduction in thymic and bone marrow output at 6 and 12 months after treatment initiation while the opposite was observed in natalizumab, that sequestrates lymphocytes in blood vessels, apart from the inflammation site. With the immunomodulatory IFNb both thymic and bone marrow output were stable within the first months, while KREC levels did not further decrease with age, contrary to what was observed in patients with fingolimod or natalizumab. With alemtuzumab, which temporarily depletes peripheral lymphocytes to induce their repopulation, only KREC levels increased following treatment and both parameters further remained stable with age (322).

In summary, the MS population is growing older, concurrently accumulating the comorbidities related

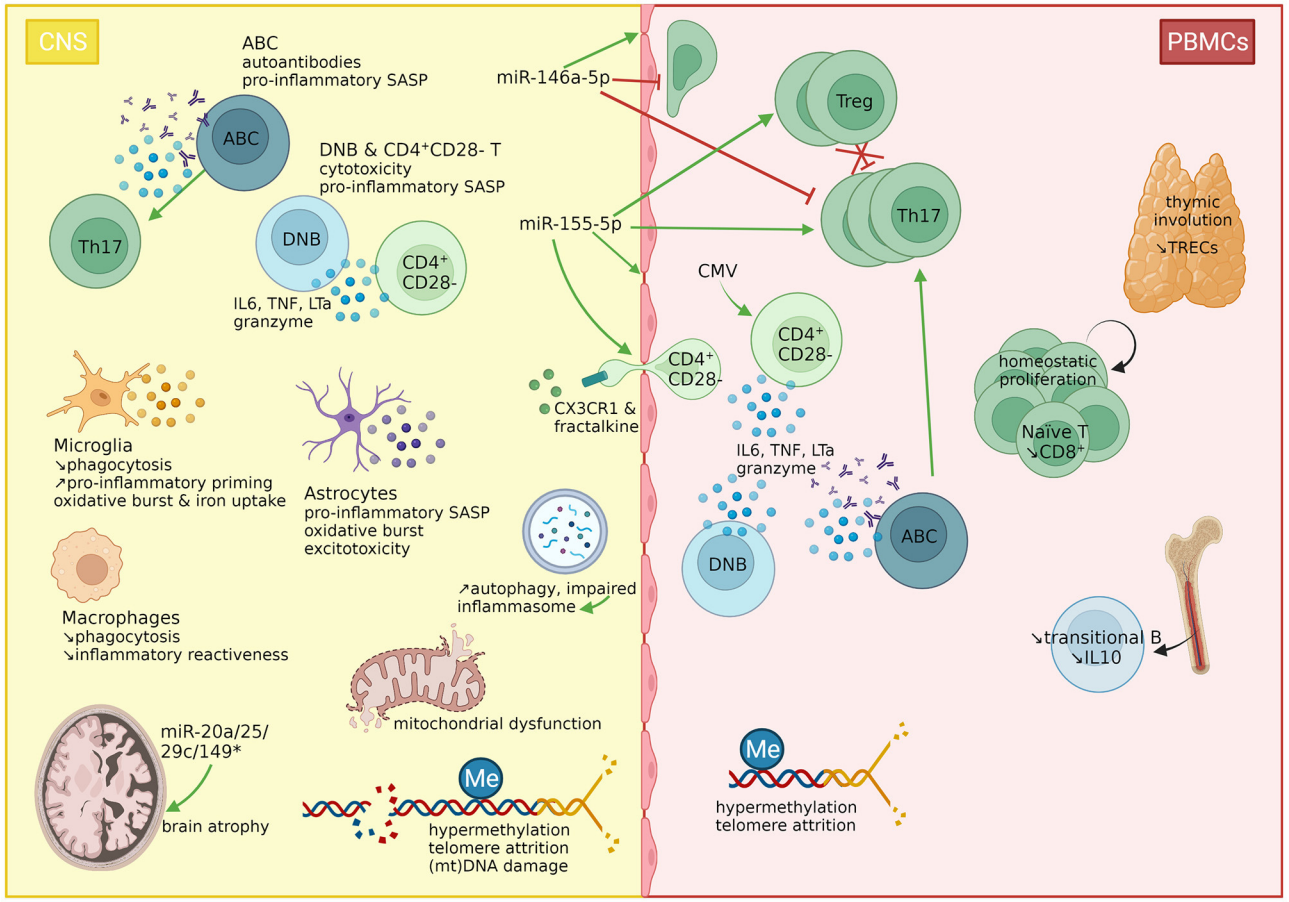

FIGURE 2 | immunosenescence features in multiple sclerosis. Features of immunosenescence have been described in MS. The thymic involution (measurable by reduced TREC levels) induces the homeostatic proliferation of T cells. However, naïve T cells rapidly differentiate into memory T cells due to antigenic stimulation. While Th17 cells, and to a lesser extent, Tregs are expanded in the periphery, the latter fail to suppress Th17 cells. A subset of $CD4^+$ T cells has lost the costimulatory signal CD28, marking the T cell exhaustion mainly through sustained CMV stimulation. These cells express the CX3CR1 receptor, favoring their migration through the BBB, as their ligand, fractalkine, has been found overexpressed in the cerebrospinal fluid (CSF). The B cell compartment is characterized by a reduction in immunoregulatory transitional B cells, but an increase in double negative B cells (DNBs) and ABCs. These three subsets ($CD4^+CD28^-$ T cells, DNBs, ABCs) have been linked to immunosenescence and detected in the CNS of MS patients. $CD4^+CD28^-$ T cells and double negative B cells produce TNFa, and granzyme B. $CD4^+CD28^-$ T cells produce also IL6, and double negative B cells produce LTa, hence corresponding to the senescence-associated secretory phenotype of these cells. ABCs produce TNFa and autoantibodies and polarize Th17 cells. In the CNS, both microglia and macrophages have impaired phagocytic properties, but while microglia are primed, macrophages lose their inflammatory reactiveness. Microglia further produce ROS and incorporate iron. Astrocytes produce also proinflammatory cytokines and ROS and release glutamate, inducing excitotoxicity. The oxidative burst causes mitochondrial dysfunction, and (mitochondrial) DNA damage. Autophagy is increased but impaired, which might induce the inflammasome. Moreover, telomeres shorten with age and disease progression. Interestingly, hypermethylation is a common feature in PBMCs from MS patients, found in several subsets as well as in brain tissues, while hypomethylation has on the contrary been linked to aging. Finally, miR-146a-5p and miR-155-5p are two major immuno-microRNAs with opposite effects on the integrity of the BBB, T cell migration and the differentiation of Th17 cells. Herein, miR-155-5p displays pro-inflammatory characteristics, but also supports the differentiation of Tregs. Four miRNAs (miR-20a/25/29c/149*) have been linked to brain atrophy. CMV, cytomegalovirus; CNS, central nervous system; CX3CR1, C-X3-C Motif Chemokine Receptor 1; B, B cell; ABC, age-associated B cell; DNB, double negative B cell; IL, interleukin; LTa, lymphotoxin A; Me, methylation; mt, mitochondrial; MS, multiple sclerosis; PBMCs, peripheral blood mononuclear cells; SASP, senescence-associated secretory phenotype; T, T cell; Th, T helper cell; TRECs, T cell receptor excision circles; Treg, regulatory T cell; TNF, tumor necrosis factor. Created with BioRender.com.

to aging. The efficacy and safety of DMTs seem to decrease with age, although robust data are still missing. DMTs might increase the risk of opportunistic infections and cancers in the elderly by changing immune cell population distributions and by affecting the functions of the immune system, hence possibly promoting certain immunosenescence features, in combination with MS-related premature immunosenescence.

Immunosenescence in Systemic Lupus Erythematosus

SLE pathogenesis is characterized by Th17 polarization, autoreactive B cells producing autoantibodies targeting nucleic acid-bound antigens and the innate immune system providing a strong IFN-I signature (323). The tissue damage in SLE, resulting in organ dysfunction (e.g., kidney, brain, lungs, cardiovascular system) corresponds to premature biological

aging. The immune dysregulation in SLE presents some features resembling immunosenescence, mainly in the adaptive immune system, but underlying mechanisms might be different. In the innate immune system, the effects of SLE and aging appear to be more divergent [reviewed by (20)].

Innate Immunity

Unlike immunosenescence, SLE-derived neutrophils primed by IFNa and autoantibodies produce more ROS and are engaged in NETosis, which causes tissue damage and partly explains the neutropenia observed in this disease (324–326). Moreover, the non-classical monocytes ($CD14^{+}CD16^{++}$), present in a decreased proportion in SLE contrarily to aging, display a reduced phagocytosis capacity, but an increased expression of TLR, TNFa and IL10 (20, 327). Macrophages contribute to SLE by their defective phagocytosis of apoptotic cells, their polarization toward a proinflammatory phenotype, and an aberrant activation of their autophagy and inflammasome machinery (328). The imbalance between the decreased immunoregulatory ($CD56^{bright}$) and the increased pro-inflammatory function ($CD56^{dim}$) of NK cells is correlated with disease activity, although their relative frequencies are unchanged, while their absolute numbers are decreased (329, 330). Moreover, increased serum levels of IFNa in active SLE parallel the frequency of IFNg-producing NK cells (330) [as seen in a TNFa/IL12-mediated viral infection response (331, 332)].

T Cells

Thymic output and TCR repertoire are reduced in SLE (333, 334). SLE patients often exhibit the key features of the immune risk phenotype, an inverted $CD4^{+}/CD8^{+}$ ratio, due to $CD8^{+}$ T cell expansion and a higher $CD4^{+}$ T cell turnover (335). Th17 and IL17-producing double negative T cells are involved in SLE pathogenesis [reviewed by (336)]. A dominant granzyme-producing $CD8^{+}$ T cell population is found in patients with severe nephritis, leucopenia, and clinically active disease (337, 338). Expanding $CD4^{+}CD28^{-}$ T cells produce IFNg in moderately active SLE and are positively correlated with the clinical disease score (339). Conversely, some autoreactive $CD8^{+}CD28^{-}$ clones secrete less IFNg and comparatively, relatively more IL10 but with impaired suppressive capacities (340). Remarkably, TCR signaling is driven by the FCgR chain in SLE rather than the TCR-zeta chain, due to its altered composition, resulting in a lower activation threshold, higher calcium influx, increased excitability and baseline stimulation [reviewed by (341)].

B Cells

Contrary to aging, there is a shift toward immature B cells in SLE, due to a two-fold increase in transitional B cells with a defective tolerance checkpoint resulting in autoreactive B cells producing autoantibodies (342, 343). Frequent cycles of B cell activation and differentiation shape peripheral B cells, marked by an expansion in switched ($IgD^{-}CD27^{+}$) memory B cells and double negative ($IgD^{-}CD27^{-}$) B cells, as well as a subset of activated memory B cells ($IgD^{-}CD27^{-}CD95^{+}CD21^{-}$), the latter being increased during disease flares (344–347). The $CD11^{high}TBET^{+}$ ABCs as well as two ABC-like subtypes, found in African American patients, have been linked to disease severity (348, 349). Double negative type 2 $CXCR5^{-}$ cells with a unique cytokine, cytokine receptor, transcription factor and signaling factor expression profile, are increased in young patients, and do not further expand with age (350).

Tregs

Data on the number and function of Tregs in SLE are very disparate, mainly due to study population and staining strategy differences. However, Tregs appear to be largely outpaced by the T and B cell activation in SLE, possibly due to the decrease in IL2 production, that mediates Treg homeostasis, and the increase in IL6 production, that induces effector T cell activation. Furthermore, the Th17/Treg ratio increases in SLE alongside the decrease of TGFb. Finally, the effect of IL10 remains unclear as it has both anti-inflammatory effects (when produced by Tregs and type 1 T-regulatory cells) and it induces autoreactive B cell proliferation (when produced by monocytes and B cells) [reviewed by (351–353)].

Inflammatory Mediators Related to Inflammaging

The binding of immune complexes induces the production of pro-inflammatory inflammaging-associated cytokines, such as TNF, IL6, and IL18 by monocytes/macrophages, but also of IFN-I by plasmacytoid DCs, and immunoregulatory cytokines IL10, IL1, and BAFF. Interestingly, they have been linked to disease activity, while CRP for instance is not [reviewed by (354)].

Proteostasis/Autophagy

Contrary to aging, autophagy is increased in SLE, as highlighted by the increased autophagy-associated markers in naïve $CD4^{+}$ T cells (355). Moreover, the autophagosome density of B cells is positively correlated with disease activity (356). However, while autoantibodies from lupus patients can induce autophagy in T cells of healthy controls *in vitro*, T cells from SLE patients are resistant to it (355).

Telomeres/Telomerase

Overall, telomere length is shorter compared to aged-matched healthy controls in SLE patients, with the reduction being even more pronounced in younger subjects, and without the typically progressive age-related decline seen during physiological aging. On the contrary, telomerase activity is increased in T and B cells. However, this fails to compensate for the accelerated telomere attrition in T cells (357, 358). Telomerase activity, but not telomere length, is positively correlated with disease activity (357). Unlike what is observed during aging, $CD8^{+}CD28^{-}$ T cells in SLE have longer telomeres, an increased telomerase activity and a preserved proliferative potential (359).

Oxidative Stress/Mitochondrial Dysfunction

Activated SLE T cells produce an excess of ROS and RNS and deplete the glutathione reserve, which leads to mitochondrial dysfunction (166, 360, 361). Oxidated DNA and mtDNA damage are correlated with the high serum

levels of cytokines (IL10, IL23, IFNa, IFNg) and chemokines (CXCL10 and MCP1) (362). Remarkably, only higher mtDNA damage levels are related to disease duration (363, 364). Moreover, peroxynitrite-modified histones, due to amino-acid nitration by RNS, induce high titers of anti-histone antibodies and UV-induced DNA damage potentially induces IFN-I (365, 366).

Epigenetics

DNA methylation levels are globally decreased in T cells from SLE patients. Herein, the hypomethylation of interferon signature genes [*i.a.* interferon regulatory factor (IRF)5, IRF7] is a hallmark of SLE pathogenesis and immune response genes are associated with chromatin remodeling (e.g., trimethylation of H3K4) (367–369). Moreover, nitration of the protein kinase C (PKC/ERK pathway) inhibits its delta catalytic activity, resulting in decreased activity of DNMT1 and thus low methylation levels in $CD4^+$ T cells allowing the transcription of *CD70*, possibly *CD11a*, and perforin (370–372). Interestingly, miR-21/29b/126/148, which are overexpressed in $CD4^+$ T cells from SLE patients, downregulate *DNMT1* (373–375), while miR-199a-5p increased splenic $CD4^+$ T cell senescence by inhibiting *SIRT1* and thus increasing the acetylation and consequently the activation of p53 (376). Upregulation of miR-7 and -30a in B cells of SLE ensures B cell proliferation, differentiation to plasma cells and antibody production (377, 378), while miR-15a activates the apoptosis of Bregs by targeting *BCL2* (379, 380). Furthermore, miR-142 downregulation by histone and DNA methylation of its regulatory region, results in the activation of T cells, hyperstimulation of B cells and suppression of Treg function (381, 382). miR-146a inhibits and miR-155 enhances IFN-I (383, 384). Finally, miR-125a stabilizes Treg-mediated homeostasis but is downregulated in SLE, and mir-31 and -34a, induced by the NF-kB pathway, target *FOXP3* (385–388).

Immunosenescence in Rheumatoid Arthritis

RA is likely due to a systemic immune dysregulation, possibly driven by DCs and macrophages that present citrullinated antigens to autoreactive T cells, inducing the production of pro-inflammatory and joint damaging mediators and causing synovial inflammation, and articular and extra-articular tissue damage (389, 390). A premature senescent phenotype of immune cells has been evidenced in RA and partly linked to its pathogenesis [reviewed by (21)].

Innate Immunity

Neutrophils are involved in generating citrullinated autoantigens that are afterwards externalized by NETosis, while anti-citrullinated protein antibodies promote NETosis. Neutrophils in joints also produce cytokines and ROS and release proteases by degranulation (391). Intermediate (non-classical) pro-inflammatory $CD14^+CD16^+$ monocytes were similarly increased in elderly subjects with atherosclerosis and middle-aged RA patients as compared to young healthy controls (392, 393). Young RA patients have also a higher frequency of $CD56^+$ monocytes, producing more TNFa, IL10, IL23, and ROS, although this is normalized by TNFa blocking therapy. Interestingly, in RA patients, the age-dependency of circulating $CD56^+$ monocytes is lost (394). Overall, macrophages drive joint inflammation in RA by secreting cytokines, chemokines and tissue degrading enzymes, activating fibroblast-like synoviocytes (FLSs) and promoting T cell infiltration and osteoclastogenesis. However, it is currently unknown how the heterogeneity between infiltrating monocyte-derived and tissue-resident macrophages might impact disease pathogenesis (395). Furthermore, in RA, the NK cells are reduced in number and functionally impaired, seen their increased production of ROS and proinflammatory cytokines, hence hindering their immunoregulatory properties (396, 397).

T Cells

The alteration in T cell homeostasis occurs early in RA and is independent of disease duration (398, 399). The proliferative capacity of $CD34^+$ HSCs in the bone marrow is reduced due to a decreased ERK signaling pathway. Both $CD34^+$ HSCs and naïve $CD4^+$ T cells from RA patients are more susceptible to apoptosis, hence reinforcing the burden on homeostatic proliferation in the periphery (398, 400–402). RA patients have a thymic output of healthy individuals aged 20–30 years older. TREC levels are already lower than normal in young RA patients (159, 399). The TCR repertoire is prematurely contracted in both naïve and memory T cells (403). The $CD4^+/CD8^+$ ratio is increased in the blood, and inverted in the synovial fluid (404). Th17 cells are possibly increased in the blood of RA patients and might be more important at early stages of the disease [reviewed by (405)]. Memory T cells are unchanged in the periphery as compared to controls. However, effector memory $CD8^+$ T cells are increased in the synovial fluid (404).

Circulating $CD4^+CD28^-$ T cells produce higher levels of TNFa and IFNg than the $CD28^+$ T cells. Their rate is correlated with disease severity and the extent of extra-articular manifestations (406, 407). These cells easily react to neoantigens as they express *de novo* NK receptors (CD56, NKG2D) in RA patients (408, 409). Interestingly, i*n vitro* generated $CD56^+CD28^-$ T cells by repeated stimulation of $CD56^-CD28^+$ T cells of young healthy donors, expressed BCL2, p53, and $p16^{INK4}$ that induce cell cycle arrest, a hallmark of cellular senescence, and activated the NF-kB pathway (410). Since they also express CX3CR1, they can migrate into the synovial fluid where FLSs express the ligand fractalkine, induced by TNFa and IFNg. This interaction activates the $CD28^-$ T cells, induces the expression of pro-inflammatory cytokines and facilitates the proliferation of FLSs and the secondary activation of T cells (411). The expansion of $CD4^+CD28^-$ T cells in RA patients has been associated with the HLA-DR4 risk factor and a TNFa polymorphism, alongside increased TNFa and IFNg production (159, 412).

B Cells

The effect of senescent B cells is not well-described in RA (21). However, transitional B cells are reduced and have

impaired functions in PBMCs of active RA (413). Moreover, double negative B cells were increased in the periphery and ABCs have been detected in the synovial fluid of RA patients (414, 415).

Tregs

Although discrepancies exist, Tregs appear to be reduced among the PBMCs in active RA, and normalized during remission as compared to controls (416, 417). In the synovial fluid they are overall increased but functionally impaired (417, 418). Interestingly, a novel subset of senescent $CD28^-$ Treg-like cells, characterized as $CD4^+FOXP3^+CD28^-$, was discovered in the blood and synovial fluid of RA patients. They express markers such as CD25, cytotoxic T-lymphocyte-associated protein (CTLA)4 and FOXP3, and also exhibit premature senescence, as shown by reduced TREC levels and an accumulation of phosphorylated gamma-H2AX (upon DNA double-strand break). Moreover, their SASP consists of high levels of pro- (TNFa, IFNg, IL2, IL4, IL17) and anti-inflammatory (IL10) cytokines. However, $CD28^-$ Treg-like cells had also impaired suppressive capacities. They could be obtained *in vitro* by stimulating $CD28^+$ Tregs with TNFa. Although $CD28^-$ Tregs numbers correlated with age, nor $CD28^-$ nor $CD28^+$ Tregs correlated with disease duration and clinical features (419).

Inflammatory Mediators/Inflammaging

Similarly to inflammaging in the elderly, RA patients have increased systemic levels of pro-inflammatory cytokines (IL6, CRP, TNFa) (420). In the synovial fluid TNFa, MMP1, and MMP3 levels were increased in early and established RA, IL10 decreased during established RA only as compared to osteoarthritis (421). Moreover, RA-derived FLSs produce more IL6, IL8, vascular endothelial growth factor, and prostaglandin E2 in response to IL1b during *in vitro* induced senescence (422). Both TNFa and IL6 play a major role in activating effector cells, inducing cytokine/chemokine/autoantibody production and tissue damage [reviewed by (389)].

Proteostasis/Autophagy

While autophagy decreases with age, it is increased in RA FLSs due to stress-induced endoplasmic reticulum hyperactivity and an elevated protein turnover, but the ubiquitin-proteasome system is impaired in RA (423, 424). This altered proteostasis may enhance inflammation.

Telomeres/Telomerase

The increased telomerase activity of HSCs is insufficient to compensate for telomere shortening (400, 401). Naïve T cells fail to induce telomerase activity following antigen priming. Telomere attrition was observed in granulocytes, naïve and memory T cells (398, 399, 425). Interestingly, telomerase activity of infiltrating cells correlated with synovial lining hyperplasia, but was independent of disease duration or severity (426). The HLA-DR4 risk factor in RA induces premature immunosenescence by accelerating telomere shortening (425).

Oxidative Stress/Mitochondrial Dysfunction

Naïve and memory T cells from RA patients have high levels of DNA double-strand breaks due to impaired DNA repair mechanisms [e.g., reduced DNA repair kinase ataxia telangiectasia mutated (ATM)] (427). Moreover, T cells isolated from RA patients enhance the activity of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), a DNA repair enzyme. The DNA-PKcs-Janus kinase-axis causes chronically cellular stress and intensifies the inflammatory activity of T cells (428). Cell-free mtDNA, released by tissue damage, was found in plasma and synovial fluid of RA patients. Interestingly, intra-articular injection of oxidized mtDNA in mice caused arthritis (429). MtDNA damage in RA (induced by TNFa and ROS) is positively correlated with macroscopic synovitis, and synovial TNFa and IFNg levels, but does not depend on age (430). Furthermore, $p16^{INK4}$ and $p16^{INK4}$-encoding genes along with IL6 could be induced in FLSs by H_2O_2 or TNFa (431). Likewise, p53 was upregulated in synovial tissues from early and late-stage RA as compared to normal synovial tissue (432). Interestingly p53 mutations, secondary to chronic oxidative stress, have been detected in RA synovial tissue and promoted clonal FLSs expansion and IL6 expression (433).

Epigenetics

Epigenetic changes in RA promote the pro-inflammatory profile involved in disease pathogenesis. Global hypomethylation, along with a decrease in active DNMT1 in the FLSs was found in RA (434). Hypomethylation in PBMCs is correlated with the disease activity score (435). The promoter gene of IL6 and TNFa is hypomethylated in PBMCs and in peripheral naïve $CD4^+$ T cells, respectively (436, 437). The IFNg locus is hypomethylated in $CD4^+CD28^-$ T cells as compared to $CD28^+$ counterparts resulting in increased expression of IFNg and TNF in the periphery and of IL17, CXCR3, CCR6 in the synovial fluid (406). Histones are globally hyperacetylated by decreased HDAC activity in RA, in particular H3 acetylation in the IL6 promoter was increased in the FLSs (438, 439). TNFa-mediated SIRT1 overexpression in FLSs induced IL6 and IL8 expression and protected cells from apoptosis (440). Moreover, miR-16 and -146a are elevated in synovial fluid, plasma and PBMCs of RA patients and are linked to disease activity (441, 442). Interestingly, HDAC downregulation restored the expression of miR-16 (443). miR-146a and -155 were upregulated in FLSs of RA, both induced by TNFa and IL1b. While miR-155 appeared to be compensatory to joint destruction by reducing MMP1/3, the role of miR-146a in FLSs is unknown, but in PBMCs it fails to properly repress *IRAK1/TRAF6* and thus the NF-kB pathway (444–446).

TABLE 1 | Comparison between immunosenescence features in aging, SLE, RA, and MS.

	Immunosenescence features	Physiological aging	MS	SLE	RA
Innate immunity	NK cells	↘ $CD56^{bright}$ ↗ $CD56^{dim}$ Impaired functions	↗ $CD56^{bright}$ in CSF (RRMS) ↗ $CD56^{dim}$ in serum (PP/SPMS) Impaired functions	↘ absolute numbers Impaired immunoregulatory function	↘ numbers Impaired immunoregulatory function
	Neutrophils	↘ NETosis/phagocytosis	↗ NETosis, ↘ apoptosis ↗ in CSF (onset/early in relapse)	↗ NETosis, neutropenia	↗ NETosis
	Monocytes	↘ classical, ↗ non-classical	↗	↘ non-classical	↗ non-classical, ↗ $CD56^{+}$
	Macrophages	↘ phagocytosis/APC function ↘ proinflammatory	Macrophages: ↘ proinflammatory (PMS) Microglia: ↗ proinflammatory	↗ proinflammatory	↗ proinflammatory
	Phagocytosis	↘	↘ (microglia & macrophages, PMS)	↘	↘
Adaptive immunity	Thymic output	↘	↘	↘	↘
	TCR repertoire	↘	↗	↘	↘
	T helper	↘ Th1, ↗ Th2 cytokines ↗ Th17	↗ Th1 ↗ Th17 ↘ Th1, ↗ Th2 cytokines with age	↘ Th1 cytokines ↗ Th17 ↗ IL17-producing DNT	↗ Th1 ↗ Th17 (in early stages)
	Memory T cells	↗	↗	↗	Unchanged T_{EM} in blood ↗ T_{EM} (CD8+) in SF
	Terminally differentiated $CD4^{+}CD28^{-}$	↗ related to CMV infection	↗	↗	↗
	$CD4^{+}/CD8^{+}$ ratio	<1	>1, especially in CSF	<1	>1 in blood <1 in SF
	Treg numbers suppressive function	↗ ≈ (vs. Th1), ↘ (vs. Th17)	≈/↗ ↘	↘ ↘	↘ in blood (active RA), ↗ in SF ↘
	Immature B cells	↘ transitional B cells, impaired function	↘ transitional B cells, impaired function Present in CSF	↗ transitional B cells	↘ transitional B cells impaired function (active RA)
	Memory B cells class-switched $IgD^{-}CD27^{+}$/unswitched $IgD^{+}CD27^{+}$	Unchanged	Unchanged in blood ↗ class-switched in CSF (adult MS) ↗ unswitched in CSF (pediatric MS)	↗ class-switched ↘ unswitched	↘ class-switched
	Double negative B cells	↗	↗ in <60 years-old, in blood/CSF	↗	↗
	ABCs	↗	↗ in <60 years-old, in blood/CSF	↗	Detected in SF
SASP	Inflammaging	IL6/8, CRP, TNFa	↗ IL6 and TNFa in serum/CSF (relapse, SP/PPMS) ↗ IL10 in serum (remission), in CSF (relapse)	↗ TNF, IL6, IL18, IFN-I ↗ IL10, IL15, BAFF	↗ IL6, CRP, TNFa (serum) ↗ TNFa, MMP1, MMP3, ↘ IL10 (SF)
Other senescence features	Oxidative stress	↗	↗↗	↗↗	↗↗
	Autophagy	↘	↗ but impaired	↗ but impaired	↗ but impaired
	Telomere length	↘	↘	↘ Except in $CD8^{+}CD28^{-}$	↘
	DNA methylation	↘	↗	↘	↘

APC, antigen presenting cells; BAFF, B cell activating factor; CMV, cytomegalovirus; CRP, C-reactive protein; CSF, cerebrospinal fluid; DC, dendritic cells; DNT, double negative T cells; IFN, interferon; IFN-I, type I interferon; IL, interleukin; MMP, matrix metalloproteinase; MS, multiple sclerosis; NET, neutrophil extracellular traps; NK, natural killer cells; PP/SPMS, primary/secondary progressive MS; RA, rheumatoid arthritis; RRMS, relapsing-remitting MS; SLE, systemic lupus erythematosus; SASP, senescence-associated secretory phenotype; SF, synovial fluid; TCR, T cell receptor; T_{EM}, effector memory T cell; Th, T helper; TNF, tumor necrosis factor; Treg, regulatory T cells. ↗, increased; ↘, decreased; and ≈, approximately equal.

A Premature Immunosenescence in Autoimmune Diseases? A Comparison Between Physiological Aging, MS, SLE, and RA

We have compared key features of immunosenescence occurring in physiological aging with changes of the immune system evidenced in MS, SLE, and RA (**Table 1**). While some features of immunosenescence are found in the 3 AIDs, others differ from physiological aging but also between them. Hence, it seems still unclear whether these findings are inherent to the disease course or causative of its pathogenesis.

In MS (**Figure 2**), innate immunity does not seem affected by senescence, at least in the early stages of the disease. However, the role of NK cells remains debated, and neutrophilic NETosis is increased contrarily to aging. Macrophages that are strongly involved in the neuroinflammatory processes in the early stages of the disease, lose their function with aging, while microglia remain highly primed. T and B cells display some immunosenescence features: $CD4^+CD28^-$ T cells, ABCs, and double negative B cells are expanded, and exhibit properties supporting autoreactivity. However, the inversion of the $CD4^+/CD8^+$ ratio is missing. Memory Tregs are increased, but their functionality is presumably reduced. In SLE, the innate immune function strikingly differs from what is observed during aging (increased NETosis, decreased non-classical monocytes, a proinflammatory macrophage shift). However, T and B cells display immunosenescence features ($CD4^+/CD8^+$ inversion, $CD4^+CD28^-$ T cell, ABC, ABC-like and double negative B cell expansion), contrarily Tregs are possibly reduced in number and function. In RA, innate immunity shows features of immunosenescence in monocytes as well as a reduced immunosurveillance by NK cells. Macrophages have reduced phagocytic properties despite actively contributing to the inflammation as do neutrophils through increased NETosis. The T cell compartment is marked by an expansion of $CD4^+CD28^-$ T cells, including functionally impaired $CD28^-$ Tregs, which are possibly involved in RA pathogenesis. The $CD4^+/CD8^+$ ratio is inversed in the synovial fluid but not in the blood. Double negative B cells and ABCs have been detected in RA, but are barely characterized.

The released inflammatory mediators (e.g., IL6, IL10, TNFa) contribute to the disease pathogenesis of all 3 AIDs and even mirror disease activity in MS. In all 3 AIDS, telomeres are shortened, except in $CD4^+CD28^-$ T cells in SLE, which consequently have a preserved proliferative potential. Mitochondrial dysfunction is increased as is observed in physiological aging, and dysregulated miRNAs are largely involved in inflammatory pathways. However, contrary to aging, autophagy is increased; but impaired. Distinctively, MS appears to feature a global hypermethylation, with distinct clusters between the disease subtypes, rather than the hypomethylation observed during physiological aging as well as in SLE and RA.

CONCLUSION

Immunosenescence encompasses functional and phenotypic changes within the immune system occurring naturally during aging. Among other features the resulting loss of self-tolerance, alongside inflammaging might be involved in the pathogenesis of AIDs. In this review, we have discussed and compared the similarities and discrepancies between hallmarks of immunosenescence in MS, SLE, and RA. Notably, cell types that are characteristic of immunosenescence and prone to autoreactivity, i.e., $CD4^+CD28^-$ T cells, ABCs and double negative B cells are expanded in MS. Although their functional features support a possible involvement in MS pathogenesis, it is currently not clear how and to which extent they contribute to the inflammatory processes in the different stages of MS. Hence, they might only reflect the consequences of chronic inflammation rather than the cause of disease. Moreover, the self-generated and self-sustained pro-inflammatory and oxidative environment within the CNS under ongoing recruitment of inflammatory and glial cells, possibly potentiates or causes premature immunosenescence. However, with disease progression the compartmentalized CNS inflammation is also governed by a distinct cellular senescence mechanism. Oxidative stress-induced mitochondrial dysfunction within CNS-resident cells progressively and irreversibly contributes to cellular and continued tissue damage, reduced remyelination capacity, impaired brain plasticity and finally loss of neuro-axonal reserves. Further research is needed to unravel the clinical relevance of these mechanisms, in relationship to immunosenescence, to improve treatments for MS at all ages and disease stages, with an acceptable risk-benefit profile.

AUTHOR CONTRIBUTIONS

OP and VvP contributed to the conceptualization of this work and to the research of the current literature on the subject. OP extensively compiled current knowledge on the subject to write and correct the first draft. VvP commented, corrected, and validated the first draft. All authors contributed to the article and approved the submitted version.

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Late-Onset MS: Disease Course and Safety-Efficacy of DMTS

Maria Chiara Buscarinu[1,2], Roberta Reniè[3], Emanuele Morena[1], Carmela Romano[3], Gianmarco Bellucci[1], Antonio Marrone[1], Rachele Bigi[1], Marco Salvetti[1,4] and Giovanni Ristori[1,2]**

[1] Department of Neuroscience, Mental Health, and Sensory Organs, Sapienza University, Rome, Italy, [2] Neuroimmunology Unit, IRCCS Fondazione Santa Lucia, Rome, Italy, [3] Department of Clinical-Experimental Neuroscience and Psychiatry, Sapienza University, Rome, Italy, [4] IRCCS Istituto Neurologico Mediterraneo (INM) Neuromed, Pozzilli, Italy

Multiple sclerosis (MS), an inflammatory demyelinating and neurodegenerative disease of the central nervous system, usually begins between the ages of 20 and 49 years, though in rare cases it is diagnosed in childhood and adolescence before the age of 18 years, or at the age of 50 years and later. When the onset of the disease occurs at 50 years or older it is conventionally defined as late onset MS (LOMS). Compared to classical MS, the LOMS is characterized by progressive course, a greater delay in diagnosis and a higher prevalence of motor disability. The older the patients, the greater is the risk of comorbidities that can negatively influence the course of the disease and can limit therapeutic strategies. To date, there is no study focused on the efficacy of Disease Modifying Therapies (DMT) in older patients with MS. The only data available are retrievable from subgroup analysis from phase-3 trials of DMT efficacy. In this work, we discuss how the aging process influences the onset, the clinical course and the therapeutic approach in LOMS.

Keywords: late onset multiple sclerosis, immunosenescence, disease modifying therapies, efficacy, safety

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***Correspondence:**
Marco Salvetti
marco.salvetti@uniroma1.it
Giovanni Ristori
giovanni.ristori@uniroma1.it

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INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disease of the central nervous system affecting million people worldwide (1). It is the major cause of non-traumatic neurologic disability in young adults (2). MS is usually diagnosed between the ages of 20 and 49 years, though in rare cases MS is observed in childhood and adolescence before the age of 18 years, or at the age of 50 years and later (3).

When the onset of the disease occurs at 50 years or older it is conventionally defined as late onset MS (LOMS). The prevalence rates of LOMS range from 0.6 to 12% (4, 5). The mean age at onset was between 53.8 and 67 years; the 25.3% with relapsing reminting (RR) MS progressed to secondary progressive (SP) MS (6).

Compared to classical MS, the LOMS is characterized by progressive course, a greater delay in diagnosis and a higher prevalence of motor disability. The older the patients, the greater is the risk of comorbidities that can negatively influence the course of the disease and can limit therapeutic strategies. To date, there is no study focused on the efficacy of Disease Modifying Therapies (DMT) in older patients with MS. The only data available are retrievable from subgroup analysis from phase-3 trials on the efficacy of disease-modifying therapies (DMT).

The advanced age exerts a great impact in the main aspects of the disease: the clinical course, the pathological and immunological processes, and the therapeutic choices. The aim of this review is discussing how the aging process influences the onset and the clinical course of MS, as well as to survey the issues for a therapeutic approach in LOMS. Since informative data on the relationship between age, MS and DMT are largely lacking, a pressing problem in clinical practice is to evaluate the safety of certain DMT in LOMS people. To examine this topic, we searched PubMed for all articles published from database inception to October 2021, with no language limitations. Keywords included: late onset multiple sclerosis; elderly multiple sclerosis; immunosenescence; disease modifying therapies.

AGE AND MS: IMMUNOSENESCENCE AND DISEASE COURSE

The weakening of the immune system associated with the natural aging (i.e., the immunosenescence) might be at least partly responsible for the transition of the disease course from an inflammatory to a neurodegenerative phenotype.

To evaluate age-related immunologic alterations in MS, Eschborn et al. compared immune signatures in peripheral blood and CSF by flow cytometry in patients with RR or primary progressive (PP) MS and respective controls (HD). The authors observed signs of premature immune aging in young patients with MS with alterations in immunoregulatory and costimulatory molecules that were comparable to those observed in elderly HDs. The characterization of major immune cell populations revealed an age-dependent decrease in the proportions of B cells and CD8+ T cells with a concomitant increase in CD4 T cells in HDs. In MS patients, these age-dependent alterations were significant for CD4+ and CD8+ T cells. In aged controls and patients, a decrease in naive CD8 T cells and a reciprocal increase in CD8 memory T cells was observed, especially in patients with PPMS compared with patients with RRMS. In an additional independent cohort, the same authors studied age-dependent alterations in immune cell composition and activation status in the CSF of patients with RR and PP disease as well as in non-inflammatory diseases. They observed an age-dependent decrease in counts of B and T cells, plasma cells and natural killer cells in patients with PPMS, but not in patients with RRMS, suggesting an age-dependent decrease in immune cell infiltration into the CSF of PPMS patients (7).

Numerous studies have demonstrated a decreased capacity for neurological repair with aging. Microglia and macrophages, innate immune cells important for central nervous system (CNS) regeneration, undergo senescence in distinct ways, that negatively impact the repair response in the aging CNS. Macrophages are less able to produce a functional pro-inflammatory response, while microglial cells exhibit an exaggerated proinflammatory response, a phenomenon referred to as microglia priming. Both aging microglia and macrophages exhibit deficits in phagocytic and chemotactic functions. Intervening with stimulation that may lead to a rejuvenation of aging macrophage/microglia may preserve neurological integrity and promote regeneration in the aging central nervous system (8).

There seems to be an increased prevalence of LOMS as well as of very-late-onset MS (VLOMS; conventionally the cases after 60 years). The rise in these forms is probably due to increased longevity during the last decades (9). In a recent work, whose aim was to compare demographic and clinical features of individuals with early onset, adult, and late onset MS, Mirmosayyeb et al. evidenced that individuals with LOMS have more frequently motor dysfunctions, sensory disturbances and visual impairments. The mean age at onset was 53.8 years and the disease affected more often the man (6).

Other works showed that these patients have increased risk of presenting an initial PP clinical onset, an earlier conversion to a SP disease, as well as earlier reaching of severe disability (10). In fact, studies involving patients with late onset reported a significantly higher disability: over 90% of the patients had an Expanded Disability Status Scores (EDSS) above 6.0 (11), with spinal demyelinating lesions and substantial spinal cord atrophy (12). Sixty% of cases became wheelchair-dependent or bedridden, with frequent accompanying symptoms, such as spasticity, sphincter and urinary disturbances, muscle aches (13).

In addition to motor decline, these patients more frequently present a marked cognitive impairment due to a high burden of cortical lesions, the presence of meningeal lymphoid follicle-like structures and a substantial increase in diffuse brain atrophy (14–18). Peculiarities in MRI of LOMS cases are a reduced chance of detecting active lesions and an increased possibility of detecting smoldering plaques (19), which are often also termed chronically active. They demonstrate lesion-specific rim activity associated with iron-laden macrophages and amplification of the oxidative injury owing to ferritin accumulation, being particularly associated with the onset of progressive disease and with the accelerated accumulation of physical disability (20).

The risk of comorbidities can negatively influence the course of the disease and can limit therapeutic strategies. Among those with the highest incidence, there were stroke and cancer. The five most prevalent comorbidities were depression, anxiety, hypertension, hyperlipidaemia and chronic lung disease. Thyroid disease and psoriasis were the most prevalent autoimmune diseases, while the tumors with the highest incidence in this MS population were the head-neck, breast and digestive system cancers (21).

The distinctive features of LOMS should be taken into consideration for the choice of treatment. The immunosenescence could in fact negatively influence the efficacy of Disease Modifying Therapy (DMT). On the other hand, some therapies could increase the risk of comorbidity or may be relatively contraindicated in these forms of MS.

AGE AND EFFICACY OF DMT

Nowadays, treatment of older patients affected by MS can be really challenging. Patients with LOMS are less frequently exposed to DMTs and consequently very little is known about the

efficacy of DMTs in this understudied old population. However, considering the increase of older people affected by MS, it seems clear that a better understanding of the characteristics of these patients and their potential response to DMTs is needed (22).

A recent meta-analysis confirms that age results an essential modifier of drug efficacy in patients with MS (23). Aging and immunosenescence in turn interact with the progressive phase of the disease that tend to compare as age increases. The role of progression may thus have a key role when we evaluate the efficacy of DMTs related to the age. Actually, during the fifth decade MS patients frequently experience a transition to a progressive disease with a shift from active inflammation to a compartmentalized inflammation and a faster accumulation of disability. This different inflammatory milieu in elderly raise doubts about the efficacy of immunomodulatory agents that are active against the peripheral inflammatory process underlying MS pathogenesis (9, 24).

To date there are no studies focusing on the efficacy of DMTs in RRMS in the old patients. The only available data are retrievable from subgroup analysis performed on 3-phase trials of DMT or from observational retrospective studies. All the clinical trials exclude patients older than 55 years. So, it could be useful to define the "old" patients those who are more than 40 years old.

A recent meta-analysis on clinical trials of DMTs for RRMS demonstrated efficacy in treating disease activity independent of age; nevertheless, the authors acknowledge that clinical trials select for patients with baseline disease activity, not representing real-world patients with RRMS, where disease activity declines with age (25).

However, the difference in age between clinical trials population and real-world data population is constantly growing. Clinical trial results seem to be not appropriate to define an age-dependent relationship with efficacy in general MS population. For this reason, further real-world studies are needed not only to define a clear relationship between DMT efficacy and age, but also the safety of DMT discontinuation (22).

Interferon-beta (IFN-β) represents the most frequently used drug in LOMS. Shirani et al. (22) performed a retrospective observational study in which they observed the relationship between IFN-β and disability progression in older RRMS: they found that IFN-β use was not statistically related with a slowing of disability progression (22) However, a *post hoc* analysis using data from a non-interventional, prospective cohort study of patients older than 40 years with MS and starting interferon beta-1b (IFNB-1b) treatment within 6 months before study entry (NCT00787657; BEACON: BEtaferon prospective study on Adherence, COping and Nurse support) demonstrated that these patients had benefits in using IFN-β during the observational period of 2 years (24).

Among the other first-line DMT, dimethyl fumarate (CONFIRM trial; NCT00451451) (26), peginterferon-β-1a (ADVANCE trial; NCT00906399) (27), and teriflunomide (TEMSO trial; NCT00134563) (28) reduced annual relapses rate (ARR) in both young and old patients (threshold of 38 years for teriflunomide and 40 years for the others); however, all these DMTs failed to reduce the risk of disability accumulation.

A *post hoc* analysis of teriflunomide clinical trials and their extensions, as well as real world studies demonstrated efficacy on clinical outcomes regardless of age and there was no increase in infection or death in older patients (29).

In AFFIRM (NCT00027300) and SENTINEL (NCT00030966) studies, Natalizumab failed to reduce progression in patients with MS older than 40 years; in this case factors such as male sex, EDSS score higher than 3.5 and fewer than 9 baseline T2 lesions turn out to be predictors of non-responsiveness (30). Fingolimod showed similar results in the FREEDOMS trial (NCT00289978), being not able to reduce disability progression and relapses in patients older than 40 years, compared to placebo (31).

Concerning second-line approaches, a *post hoc* analysis from the randomized CARE-MS (NCT00530348, NCT00548405) trials showed that alemtuzumab did not show different efficacy in young and old patients, evaluating both inflammatory activity and disability accumulation (32).

Among the more recent DMTs, Ocrelizumab, a B cell-depleting anti-CD20 antibody, failed to reduce ARR in patients older than 40 years (33). Ozanimod and siponimod, that are second-generation sphingosine-1-phosphate receptor modulators (selectively directed against 1,5-S1PR), showed different effects on MS measure outcomes: ozanimod failed to reduce both relapses and disability progression. (NCT02047734; NCT02294058), while siponimod resulted the first DMT that showed an efficacy to reduce disability accumulation in SPMS (NCT01665144) (34).

Disability progression represents the hardest challenge in MS management. In MS progressive forms, the neurodegeneration processes are strictly connected with the decreases of CNS capacity to remyelinate with age. In particular, the failure of oligodendrocyte precursor cells (OPCs) to differentiate into myelinating oligodendrocytes represents a major key in this process. Trial with metformin showed potential to induce maturation of OPCs and following remyelination in aged rodents (35). The cellular milieu surrounding axons, myelin sheaths and OPCs, has a key role to promote remyelination. In particular, microglia and macrophages are responsible for the phagocytosis process to eliminate myelin debris, which have a negative impact in restoring myelination and OPCs maturation. Niacin or vitamin B3, upregulating CD36 expression on microglia, promote myelin debris elimination, representing an interesting therapeutic strategy in chronic forms of MS (36). Also the senolytic drug class, such as the tyrosine kinase inhibitor dasatinib and quercetin, have been shown to potentially decrease the accumulation of senescent cells in older mice and to represent a therapeutic option for age-related pathophysiology of MS (37).

In the context of the rejuvenation research, the recovery of thymus functions may reduce the defects in negative selection and in the generation of Treg cells. An age-related IL-7 decrease is shown with a consequent thymus involution (38). Proposed treatment with IL-7 results in a higher number of recent memory CD8+ T cells growth (39). Likely, IL-22 is involved in thymopoiesis and treatment with this molecule has been shown to enhance thymic recovery (40). Finally, reduce the plethora of stimuli which led to recurrent inflammatory activations, as common infections of influenza virus and CMV,

maybe another approach to reduce immunosenescence process. In this context, specific vaccination could be used to prevent persistent stimulation of the immune system, reducing the impact of peripheral inflammaging and potential triggers MS reactivation (41).

AGE AND SAFETY OF DMT

The age-related changes that take place in the immune system, a process known as immunosenescence generally result in a higher susceptibility to infections, a reduced response to vaccines and a higher prevalence of autoimmunity and neurodegenerative disorders. These processes may affect the safety of DMT, so that potentially severe adverse events are more common in elderly patients.

Data from elder MS patients are limited and of special interest in view of the fact that an increasing proportion of patients, often after long-term management of their disease, are now in the higher age groups: In 2004, an analysis of data from a large MS registry in the United States revealed that ∼14% of patients with MS were ≥65 years of age (9). The clinical trials of disease-modifying therapies for RRMS were not designed to assess efficacy in aging patients. In fact, the pivotal clinical trials of the most widely used DMT specifically excluded individuals aged >45 years (glatiramer acetate), >50 years (natalizumab and alemtuzumab) and >55 years (IFNβ1a, dimethyl fumarate, fingolimod and teriflunomide). A currently recruiting phase III study of ocrelizumab in patients with progressive MS will have the highest upper age limit (65 years) used thus far in MS clinical trials.

There are two main serious adverse events caused by the long-term use of DMT in elderly: infections, such as progressive

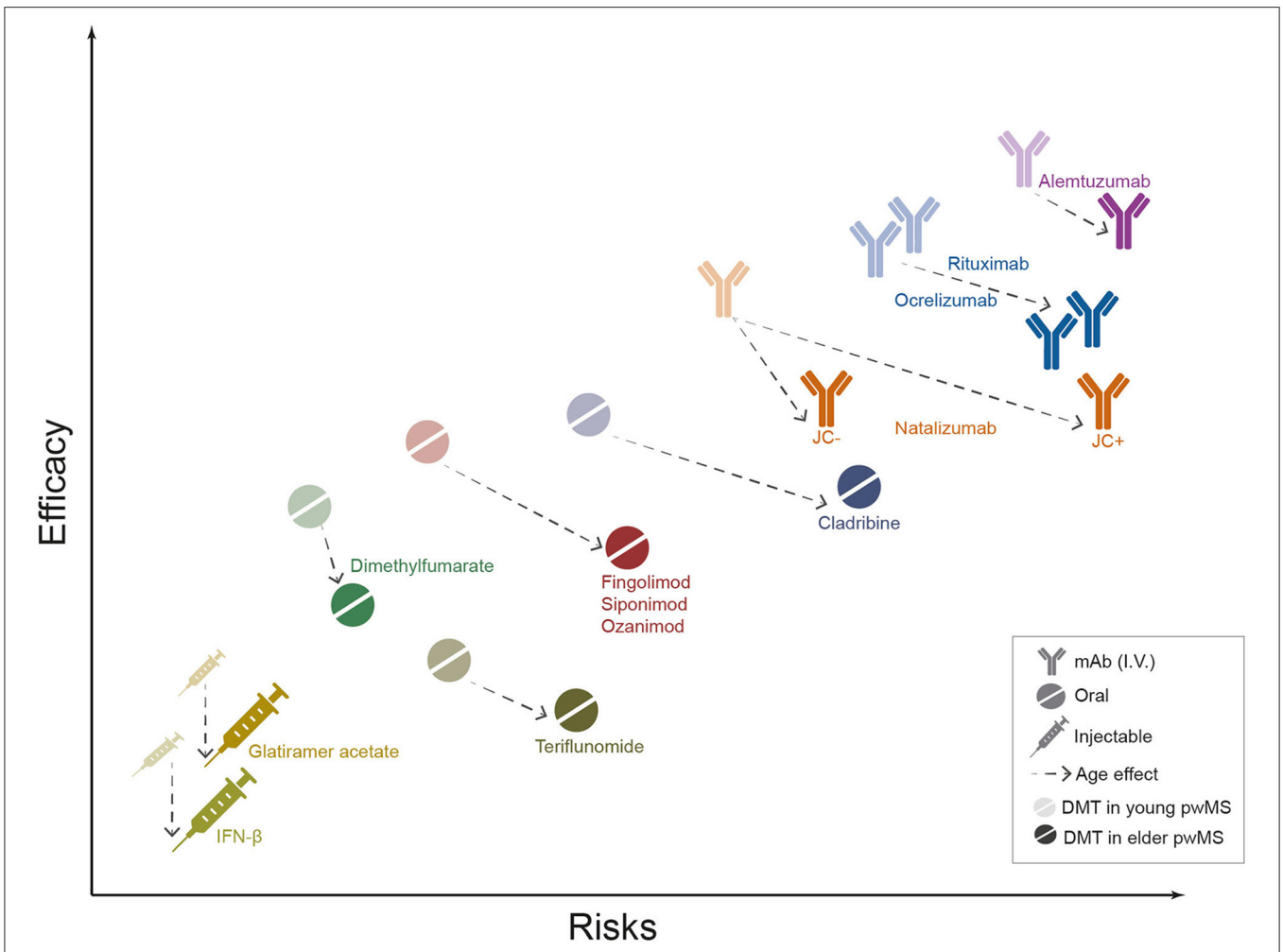

FIGURE 1 | Safety and efficacy of MS DMTs in elder people with MS (pwMS). The graph shows the position of currently used MS drugs in terms of efficacy (*y axis*) and safety (expressed as growing risk burden, *x axis*). Semi-transparent icons represent DMTs effects in younger pwMS, while solid icons display the estimated effects in elderly. Dotted arrows illustrate the estimated effect of age on DMT safety and efficacy. The drugs' profiles are based on the authors' judgment, educed from the interpretation of currently available literature data. mAbs, monoclonal antibodies; I.V., intravenous administration; IFN-B, Interferon β.

multifocal leukoencephalopathy (PML), and the potential DMT-induced cancer risk. The age-induced immunosenescence and the loss of lymphocyte functional capacity may increase the risk for occurrence of PML in MS patients treated with second-line DMTs.

The seropositivity to John-Cunningham virus (JCV), a known risk factor for PML, grows with age: an average of 10.8% conversion per year was reported (42). PML is a rare but potentially fatal complication of different DMTs. Prosperini et al. investigated if age at treatment start affects the time to onset of natalizumab-related PML. The authors showed that patients older than 50 years had a more than doubled-increased risk for an earlier PML onset (HR = 2.11, $p = 0.006$) (43). Along the same line, Jin Nakahara et al. describe 3 of the 21 registered cases of fingolimod-associated PML (without a previous natalizumab therapy) and all the patients were older than 45 years (44). In another work by Berger et al., ten out of 15 fingolimod-related PML patients were older than 50 years. In this series the patients were ∼10 years older compared with those with natalizumab-associated PML (45). Higher age may also constitute a risk factor for the rare occurrence of other opportunistic infections, such as cryptococcal meningitis during fingolimod therapy.

Teriflunomide is a once-daily oral immunomodulator approved after trials with an age limit of 55 years. A non-interventional study with teriflunomide (TAURUS-MS I) included a large cohort of real-world MS patients in Germany with data derived from 1,128 patients: 558 (49.5%) patients were above 45 years old; 131 patients in the age group >55–65; and 19 patients over 65 years old. The number of patients with AEs was lowest in patients aged 26–35 years (29.2%); serious AE were 7.7% in patients aged 26–35 years and 15.0% in patients aged 46–55 years; the rate of discontinuation was higher in patients >45 years (62.9%) (46).

Changes in serum Ig levels have been reported in MS patients (47). They can be exacerbated when B-cell depleting drugs are used as DMT. In fact reduced blood concentration of IgG, IgM, and/or IgA is known to occur in patients treated with B-cell–depleting therapy (secondary antibody deficiency), including ocrelizumab. In in the Pivotal Phase III Trials of Ocrelizumab a reduction in serum Ig levels was observed, at an approximate mean rate of 3–4% per year for IgG (48). The reduced serum IgG concentrations may lead to false-negative results in JCV antibody index test in patients treated with anti-CD20 therapies (49).

Overall, these data suggest caution in DMT choice for elder patients to prevent the high risk of serious infection and the possibility of underestimating the PML risk (**Figure 1**).

Age above 50 in general are associated with increase in incidence rates for many types of cancer in the general population. Prosperini et al. did a meta-analysis to investigate how age could influence safety in MS patients under DMTs. They demonstrated that the interaction of age with depleting drugs (alemtuzumab, cladribine, and ocrelizumab) explained ∼23% of the variance in neoplasm rate. The authors also estimated a higher neoplasm rate in patients treated with depleting agents compared to patients taking other DMTs above an average age of 45 years (50).

FUTURE PERSPECTIVES

Overall, informative data on the relationship between age, MS and DMT are largely lacking. Immunosenescence, nature of the DMT and age at onset of MS interact each other, challenging the possibility of designing studies aimed at disentangling the underlying mechanisms of the interplay. Real-world data and post-marketing surveillance are certainly of interest considering the fact that many patients who started DMT over the last decades are currently older than the age limits usually used in clinical trials.

A pressing problem in clinical practice is the safety of certain DMT in LOMS people: especially the depleting drugs seem to pose older subjects at higher risk of serious infections or cancer. This topic warrants further study specifically designed to quantify the risk and to disclose the better strategies to minimize such a risk.

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All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Aging With Multiple Sclerosis: Age-Related Factors and Socioeconomic Risks

Malthe Faurschou Wandall-Holm[1]*, *Mads Albrecht Andersen*[1], *Mathias Due Buron*[1] and *Melinda Magyari*[1,2]

[1] Danish Multiple Sclerosis Registry, Department of Neurology, Copenhagen University Hospital—Rigshospitalet, Glostrup, Denmark, [2] Danish Multiple Sclerosis Center, Department of Neurology, Copenhagen University Hospital—Rigshospitalet, Glostrup, Denmark

Background: Studies have demonstrated an increasing mean age of the population with multiple sclerosis (MS). The association between increased age and socioeconomic outcomes has been investigated sparsely.

Objective: The purpose of this study is to describe the demographic and socioeconomic status of the current Danish population of patients with MS according to age and to assess the age-related risks of no income or losing all income from earnings or receiving disability pension.

Methods: The nationwide population-based Danish Multiple Sclerosis Registry provided data linked with the Danish Income Statistics Register and the Danish Rational Economic Agents Model (DREAM) database. The prevalence of socioeconomic milestones of the current MS population was compared with healthy controls and the risks of reaching socioeconomic milestones were assessed using cause-specific Cox models and cumulative incidence functions compared to healthy controls.

Results: The current Danish population of patients with MS of working age (18–65 years of age) consists of 11,287 patients, of which 29.3% was older than 55 years. In 2018, 38.0% of all patients and 18.9% of controls had no income from earnings, whereas 30.5% of all patients and 7.7% of controls received disability pension. The risk of losing all income from earnings was higher for patients with MS with a hazard ratio (HR) peaking at of 4.0 (95% CI, 3.8–4.2) for the ages of 45–54 years. The risk of receiving disability pension was much higher for patients with MS peaking at a HR of 22.6 (95% CI, 20.9–24.4) for the ages of 25–34 years. Likewise, the absolute risks of both outcomes were higher for the patients with MS at all ages.

Conclusion: Danish patients with MS are at a higher risk of losing all income from earnings and at a much higher risk of receiving disability pension compared with healthy controls.

Keywords: multiple sclerosis, aging, age-related risks, age-related factors, socioeconomic, socioeconomic outcomes

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***Correspondence:**
Malthe Faurschou Wandall-Holm
malthe.faurschou.wandall-holm@regionh.dk

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INTRODUCTION

Multiple sclerosis (MS) is considered a disease of the young adult, although numerous studies have reported an increasing mean age of the population with MS, and an increasing incidence of the elderly (1, 2).

The proportion of patients on disability pension and without income has been shown to increase drastically after disease onset (3, 4), and the work ability after a diagnosis of MS has been shown to be reduced (5). The socioeconomic burden of MS on society is substantial and appears more than 8 years before diagnosis with productivity decreases and social benefit payments making up the majority of this burden (6, 7). On an individual level, these consequences have negative implications for both mean income (3), but also the sense of personal contribution to society.

Aging in general is linked to challenges concerning physical and mental abilities. For patients with MS, these challenges are seen much earlier in life than for the rest of the population (8). A recent Danish study has reported an increased incidence of MS most pronounced at later ages of onset (1), and previous studies have found that older age at onset is associated with a later assignment of irreversible disability levels (9). However, the interplay between age and socioeconomic outcomes has not been extensively investigated.

The aim of this study was to investigate the association between age and socioeconomic decline in patients with MS in Denmark. We chose two reliable outcomes reflecting the functional capacity of the patient (10), no income or loss of income and disability pension, and compared prevalence, hazard ratios (HRs), and cumulative incidence across age groups with the background population of Denmark.

MATERIALS AND METHODS

Study Design and Data Sources

We conducted a Danish nationwide observational study that consists of two parts: a cross-sectional study and a longitudinal study. Clinical data were obtained from the population-based nationwide Danish Multiple Sclerosis Registry (DMSR) (11) with information on patients with MS dating back to 1948. Demographic and socioeconomic data were obtained from national population-based registers: the Population Statistics Register (12), the Income Statistics Register (13) (ISR), and the Danish Rational Economic Agents Model (DREAM) (14). The primary outcomes were obtained from ISR and DREAM. The ISR contains data on income of all Danish citizens on an annual basis. DREAM contains data on all social transfer payments on a weekly basis. The unique personal identification code provided to all Danish citizens (12) enabled individual cross linkage between registers.

Cross-Sectional Study

Study Population

The reference point in time of the cross-sectional study was January 1, 2019. All patients diagnosed with MS at January 1, 2019 were eligible for inclusion. The diagnosis of MS was made according to the Poser criteria before 2005 and the McDonalds criteria and subsequent revisions after 2005. To be included in the study, patients had to be alive, living in Denmark and be between 18 and 65 years of age (considered working age) at the reference point.

Disease duration was calculated as the time in years between the onset of MS (first clinical symptom) and January 1, 2019. The Expanded Disability Status Scale (EDSS) score was defined as the latest EDSS score within 2 years of the reference point. The MS phenotype was categorized as either relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), or primary progressive MS (PPMS), and unspecified, assessed by a neurologist. Current treatment was categorized as receiving no treatment or with a disease modifying therapy (DMT) of moderate efficacy (azathioprine, dimethyl fumarate, glatiramer acetate, interferon-β, methylprednisolone cycles, peginterferon β-1a, and teriflunomide) or high efficacy (alemtuzumab, cladribine, daclizumab, fingolimod, hematopoietic stem cell transplantation, methotrexate, mitoxantrone, natalizumab, ocrelizumab, ofatumumab, rituximab, and treosulfan) at the reference point. Classification of DMTs as either moderate or high-efficacy was based on the ability to reduce relapse rates, reduction in MRI disease activity and disability accumulation (15).

Treatment coverage was calculated as years spent as either untreated, treated with a DMT of moderate efficacy or treated with a DMT of high efficacy divided by the disease duration.

Patients were grouped by age at the reference point into five categories: 18–24, 25–34, 35–44, 45–54, and 55–64. The intervals were chosen to reflect gradual changes in work ability during life.

Outcomes

No income was defined as having no income from personal earnings (including short-term sickness benefits) in the year of 2018 in the ISR. Disability pension was defined as having one or more transfer payments labeled "disability pension" in the year of 2018 in the DREAM register.

Statistical Analysis

Clinical characteristics were displayed as frequencies with corresponding percentages, mean values, and standard deviations (SD) or median values with interquartile ranges (IQR) as appropriate. The amount of missing data is displayed for each variable. To perform comparative analysis, we matched every included patient on sex and exact age as floored integers in a one-to-five manner. Controls were drawn from a random comparator sample containing 25% of the entire Danish population (excluding patients with MS). Prevalence ratios with confidence intervals (CIs) were calculated using a generalized linear model with a binomial distribution and a logarithmic link function. The calculation of a prevalence ratio is similar to that of a relative risk, but relative risk is a misnomer in the cross-sectional setting. There were no known missing data for outcomes.

Longitudinal Study

Study Population

Patients with MS and all subjects in the 25% random sample of the Danish population were eligible for enrollment into the study population from January 1, 1992 to January 1,

2019. The period was chosen due to outcome availability in DREAM and ISR. Inclusion criteria were an age between 18 and 65 during the study period (to be at risk for the study outcome), being alive at January 1, 1992 (or born later), being an inhabitant in Denmark in January 1, 1992 (or born with Danish citizenship later), and not having received disability pension before enrollment (once granted, disability pension is considered permanent in Denmark). Patients diagnosed with MS during follow-up contributed risk time in both groups, changing status on the day of diagnosis. Diagnostic criteria for MS were according to the Poser criteria until 2005 and subsequently the McDonald criteria and their following revisions.

Outcomes

Loss of income was defined as the 2nd year without income for 2 consecutive years after having had at least 1 year with an income, identified in the ISR. This composite outcome was chosen, because we wanted loss of income to represent a weakening association with the labor market, and not a fluctuation due to temporary life situations: sick leave, leave of absence, long-term travels, etc. Disability pension was defined as the first occurrence of a transfer payment labeled "disability pension" in the DREAM register.

Statistical Analysis

The HRs and corresponding 95% CIs were assessed using cause-specific Cox regression models. The models used age as the underlying timescale (16). Subjects were entered into the model in a left truncated manner, only contributing risk time while observable. Using age as the timescale automatically ensured adjustment of the models for age. To account for the time dependency of exposure, subjects contributed risk time in the five age categories: 18–24, 25–34, 35–44, 45–54, and 55–64, changing categories as they aged during follow-up. Follow-up ended at the first occurrence of either a censoring event (death, emigration, turning 65 years old, being diagnosed with MS for the reference group, or end of follow-up) or an event (loss of income or disability pension, respectively). Exposure status (patient or control) was handled in a time-varying manner. Patients who were diagnosed during follow-up contributed risk time as controls until diagnosis and as patients after.

For the assessment of the absolute risks of losing all income from earnings or receiving disability pension, we fitted the same data using a non-parametric estimator of the cumulative incidence function taking competing risks into account (17). Death, emigration, and turning 65 years old were registered as competing risks, while developing MS (for the reference group) or not having reached an event by the end of follow-up was registered as censored. Exposure status changed as described in the previous paragraph.

The amount of missing data on outcomes was negligible (<1%), and analysis was performed on a complete case basis.

Ethics, Approval, and Data Access

Observational register-based studies do not require informed consent or approval from the ethical committee in Denmark. The study was approved by the Danish Data Protection Agency. Danish data regulations dictate that access to data can only be obtained upon qualified request and approval by the Danish Data Protection Agency and the Danish Multiple Sclerosis Group.

Individual level data were pseudonymized. Table cells containing values representing data from <5 subjects (and neighbors allowing crosscell calculations) were censored due to Danish GDPR regulations. Data management and statistical analysis were performed on secure servers hosted by Statistics Denmark (18). All analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Cross-Sectional Study

A total of 27,837 patients in the DMSR were assessed for eligibility. Following exclusion of ineligible patients, 11,287 remained in the final population (**Figure 1A**). All variables were assessed in relation to the reference time point, January 1, 2019, as described in the Method section. The male-to-female ratio was ∼1:2 in all age groups. The mean age at onset of the entire population was 32.8 years (SD, 9.6 years), and the median disease duration was 13.0 years (IQR, 13.5). The median EDSS score of the entire population was 2.0 (IQR, 2.0). We found an increase in median EDSS scores of 0.5 per age group (apart from the last, that increased by 1), starting at 1.0 (IQR, 2.0) among the 18–24-year age group and ending at 3.5 (IQR, 3.50) among the 55–64-year age group. The RRMS phenotype was predominant in the two youngest age groups, making up 89.6–89.9% of cases, while only being 54.9% of the cases in the oldest age group. In total, RRMS accounted for 74.8% of all patients in the population, whereas 7.7% had SPMS, 6.7% had PPMS, and 10.8% had an unspecified phenotype. The patients with an unspecified phenotype were older with a mean age of 52.7 years (SD, 9.6 years) and the majority, 85%, did not receive treatment.

At the reference point in time, 61.1% of the total population received treatment with a DMT, with 57.6% of these on DMT of moderate efficacy and 42.4% on DMT of high efficacy. Among patients with RRMS, a total of 73.8% received a DMT, with 57.5% of these on a DMT of moderate efficacy and 42.5% on a DMT of high efficacy.

For the RRMS population, the mean proportion of the disease duration without treatment was 0.45 (SD, 0.31), whereas 0.40 (SD, 0.31) was covered by drugs of moderate efficacy and 0.14 (SD, 0.24) by drugs of high efficacy. Results for the individual age groups can be found in **Table 1**.

For the comparative analysis, cases were matched with controls in a 1:5 ratio on sex and exact age, which yielded 56,435 controls. The percentage of patients having no income from earnings ranged from 30.0% in the 18–24-year age group to 54.3% in the 55–64-year age group, whereas for controls, it was 17.8 and 24.3%, respectively. In total, 38.0% of patients and 18.9% of controls had no income from earnings in 2018. The prevalence ratio peaked between the 55–64-year age groups at 2.2 (95% CI, 2.1–2.3), whereas the smallest prevalence ratio was found in the 25–34-year age groups at 1.5 (95% CI, 1.4–1.7).

The percentage of patients on disability pension was found to be increased from 2.0% in the youngest age group to 53.1%

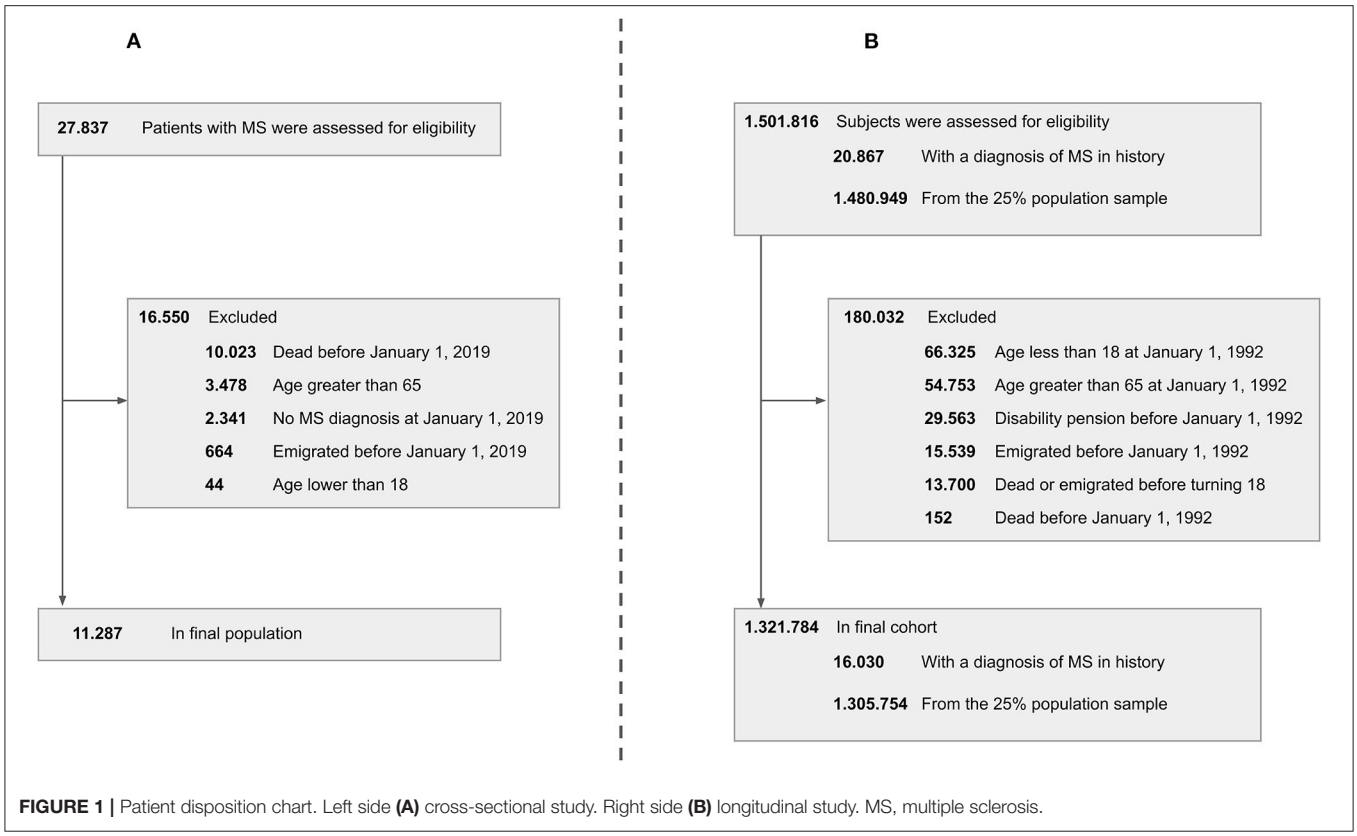

FIGURE 1 | Patient disposition chart. Left side **(A)** cross-sectional study. Right side **(B)** longitudinal study. MS, multiple sclerosis.

in the oldest, whereas the controls were found to be increased correspondingly from 1.0 to 13.8%. In total, 30.5% of patients and 7.7% of controls were on disability pension. The prevalence ratio was highest between the 35–44-year age groups at 5.4 (95% CI, 4.8–6.1) and lowest between the 18–24-year age groups at 2.0 (95% CI, 0.6–6.3), note the overlap of one in the confidence interval.

The results can be found in **Table 2**.

Longitudinal Study

A total of 1,501,816 subjects were assessed for eligibility. Following exclusion of ineligible subjects, 1,321,784 remained in the final population (**Figure 1B**), of which 16,030 were patients with MS (at some point in the study period) and 1,305,754 were controls from the 25% random sample of the Danish population.

For the loss of income from earnings-analysis, total follow-up time was 107,243 person years for the patient group and 19,828,947 person years for the control group with a mean follow-up of 8.1 and 15.0 years, respectively. We observed 5,958 events in the patient group and 352,994 events in the control group. The highest HR of 4.0 (95% CI, 3.8–4.2) was found in the 45–54-year age group, whereas the lowest at 2.2 (95% CI, 2.1–2.3) was found in the 55–64-year age group.

For the disability pension-analysis, total follow-up time was 95,533 person years for the patient group and 21,300,413 person years for the control group with a mean follow-up of 6.8 and 16.1 years, respectively. We observed 5,347 events in the patient group and 98,200 events in the control group. The highest HR of 22.6 (95% CI, 20.9–24.4) was found in the 25–34-year age group, whereas the lowest at 5.5 (95% CI, 5.2–5.9) was found in the 55–64-year age group. Results from both Cox regression analyses are presented in **Table 3**.

The absolute risks of losing all income from earnings or receiving disability pension as a function of age are displayed in **Figures 2**, **3**, respectively.

DISCUSSION

In this study, we describe a nationwide population of patients living with MS in Denmark and their socioeconomic status measured by loss of income from earnings and disability pension according to age categories.

We found that the Danish population alive and of working age consists of 11,287 patients with MS, of which approximately two-thirds are female, similar to what is reported in other populations (19). The mean age at onset was around 33 years with a median disease duration of 13 years—not surprisingly, both are increasing with age. We found a median EDSS score of 2 for the whole population, ranging from 1 for the youngest age group to 3.5 for the oldest. This was expected since the EDSS score is known to be highly age-dependent due to disability accumulating over time (9).

Further, elderly patients tend to have increased activity of neurodegenerative pathways and less effective neuro-repair processes compared with younger

TABLE 1 | Clinical characteristics of the current population with MS in Denmark.

Age	**18–24** ***n* = 197**	**25–24** ***n* = 1,310**	**35–44** ***n* = 2,695**	**45–54** ***n* = 3,779**	**55–64** ***n* = 3,306**
Female, *n* (%)	131 (66.5)	901 (68.8)	1,876 (69.6)	2,637 (69.8)	2,235 (67.6)
Age at onset, mean (SD)	18.10 (3.15)	23.70 (4.4)	29.3 (6.4)	34.4 (8.2)	38.5 (10.2)
Disease duration, median (IQR)	3.5 (3.5)	6.0 (6.0)	10.50 (10.0)	14.50 (13.0)	19.5 (16.0)
EDSS score, median (IQR)	1.0 (2.0)	1.5 (1.5)	2.0 (2.0)	2.5 (2.0)	3.5 (3.5)
Phenotype, *n* (%)					
RR	177 (89.8)	1,174 (89.6)	2,334 (86.6)	2,936 (77.7)	1,816 (54.9)
PP	9 (4.6)	65 (5.0)	132 (4.9)	218 (5.8)	335 (10.1)
SP	CENS	CENS	71 (2.6)	296 (7.8)	498 (15.1)
Unspecified	CENS	CENS	158 (5.9)	329 (8.7)	657 (19.9)
Treatments grouped by efficacy, all patients, *n* (%)					
No DMT	20 (10.2)	299 (22.8)	696 (25.8)	1,390 (36.8)	1,985 (60.4)
Moderate efficacy	81 (41.1)	507 (38.7)	1,021 (37.9)	1,432 (37.9)	935 (28.3)
High efficacy	96 (48.7)	504 (38.5)	978 (36.3)	957 (25.3)	386 (11.7)
Has received treatment (If "No DMT" above), *n* (%)	16 (80.0)	216 (72.2)	449 (64.5)	793 (57.1)	797 (40.2)
Treatment efficacy, patients with RRMS, *n* (%)					
No DMT	15 (8.5)	237 (20.2)	507 (21.7)	761 (25.9)	689 (37.9)
Moderate efficacy	73 (41.2)	456 (38.8)	931 (39.9)	1,307 (44.5)	813 (44.8)
High efficacy	89 (50.3)	481 (41.0)	896 (38.4)	868 (29.6)	314 (17.3)
Treatment coverage, patients with RRMS, mean (SD)					
Untreated	0.37 (0.27)	0.41 (0.29)	0.42 (0.30)	0.45 (0.31)	0.52 (0.33)
Moderate efficacy	0.36 (0.32)	0.38 (0.30)	0.40 (0.31)	0.42 (0.31)	0.40 (0.32)
High efficacy	0.27 (0.32)	0.21 (0.28)	0.18 (0.25)	0.13 (0.25)	0.08 (0.18)

CENS, Censored due to small cell values; EDSS, Expanded Disability Status Scale; RR, relapsing remitting; PP, primary progressive; SP, secondary progressive; DMT, disease modifying therapy; SD, standard deviation.

TABLE 2 | Prevalence and prevalence ratios for socioeconomic outcomes according to age groups from cross-sectional analysis.

Age, years	**Events/patients (*n*/*n*, %)**	**Events/controls (*n*/*n*, %)**	**Prevalence ratio (95% confidence interval)**
No income from earnings in 2018			
18–24	59/197 (30.0%)	175/985 (17.8%)	1.7 (1.3–2.2)
25–34	347/1,310 (26.5%)	1,138/6,550 (17.4%)	1.5 (1.4–1.7)
35–44	713/2,695 (26.5%)	2,073/13,475 (15.4%)	1.7 (1.6–1.9)
45–54	1,376/3,779 (36.4%)	3,285/18,895 (17.4%)	2.1 (2.0–2.2)
55–64	1,796/3,306 (54.3%)	4,015/16,530 (24.3%)	2.2 (2.1–2.3)
Receiving disability pension in 2018			
18–24	4/197 (2.0%)	10/985 (1.0%)	2.0 (0.6–6.3)
25–34	98/1,310 (7.5%)	113/6,550 (1.7%)	4.3 (3.3–5.6)
35–44	523/2,695 (19.4%)	484/13,475 (3.6%)	5.4 (4.8–6.1)
45–54	1,292/3,779 (34.2%)	1,456/18,895 (7.7%	4.4 (4.2–4.7)
55–64	1,754/3,306 (53.1%)	2,285/16,530 (13.8%)	3.8 (3.7–4.0)

patients—processes that are known to be significant determinants of disability accumulation in MS (20, 21).

The distribution of MS phenotypes was found to be almost exclusively RRMS for the youngest patients, whereas patients in the oldest age group of 55–64 years had a higher prevalence of progressive phenotypes: PPMS (10.1%), RRMS (55.0%), and SPMS (15.0%). Obviously, SPMS is more frequently seen at higher ages as it takes time for the disease to progress to the secondary progressive phase (21), but the incidence of PPMS has also been shown to increase at higher ages of disease onset (22). This is especially prevalent in male patients, who more often present with PPMS and a higher age at onset (22).

Regarding the use of DMT for patients with RRMS, we saw that high efficacy treatment was most widely used in the younger patients, and the proportion of moderate efficacy treatment

increased with increasing age. At the same time, there was an increase of not being on treatment with increasing age. In the four youngest age categories, the increase was modest, while changing category from 45–55 to 55–64 saw a relative increase of 46.2% of patients being untreated. The mean proportion of the disease duration covered by treatment increased with decreasing age from 48% among the 55–64 years old compared to 63% among the 18–24 years old. The most likely explanation for this difference is earlier diagnosis and subsequently earlier treatment starts, increased availability of treatments and a more aggressive therapeutic approach to disease management.

TABLE 3 | HRs for socioeconomic outcomes according to age groups from longitudinal analysis.

Age, years	Hazard ratio (95% confidence interval)
Loss of all income for 2 consecutive years	
18–24	3.6 (3.0–4.3)
25–34	3.0 (2.8–3.2)
35–44	3.1 (3.0–3.3)
45–54	4.0 (3.8–4.2)
55–64	2.2 (2.1–2.3)
Receiving disability pension	
18–24	19.5 (15.3–24.8)
25–34	22.6 (20.9–24.4)
35–44	12.1 (11.4–12.7)
45–54	7.9 (7.5–8.3)
55–64	5.5 (5.2–5.9)

The data illustrate that the aging patient group represents a clinical challenge with regard to treatment. A quarter of the population in the age group of 55–64 years display a distinct progressive phenotype that, despite recent advances in therapeutical options (23, 24), are still mainly left untreated. Among the patients of the highest age group still clinically regarded as having a relapsing remitting phenotype, we see a treatment drop-off accelerating with increasing age. The reason for the decrease in DMT use has not been extensively investigated and is most likely a combination of overlapping explanations, such as contraindications, patient preference, progression, or stable disease without relapses which has poor evidence on treatment effectiveness in the aging MS population (25).

In the clinical setting, no distinct event or biomarker indicates a progression from the relapsing remitting to the secondary progressive phenotype, rather it is a gradual process that at some point reaches a diagnostic threshold. The transition implies an underlying shift in pathological pathways from peripherally induced acute inflammation to chronic inflammation and neurodegenerative processes (26, 27). Since the current arsenal of treatment options for MS is targeting acute inflammation, the disease becomes increasingly treatment refractory. The combination of perceived reduced disease activity combined with less efficacy of available treatments might make the clinician and patients less prone to choose treatment. Another driver could be a higher risk of adverse events in the elderly population (25, 28).

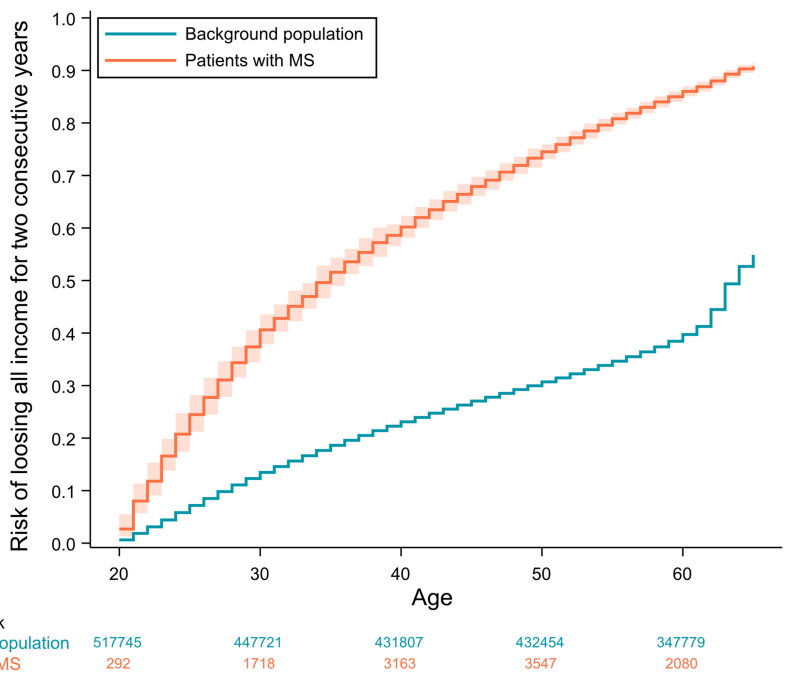

FIGURE 2 | Cumulative incidence curves of losing all income for 2 consecutive years with 95% confidence intervals adjusted for competing risks (age ≥ 65, emigration or death). Confidence intervals for the background population are not visible due to values very close to the incidence estimates.

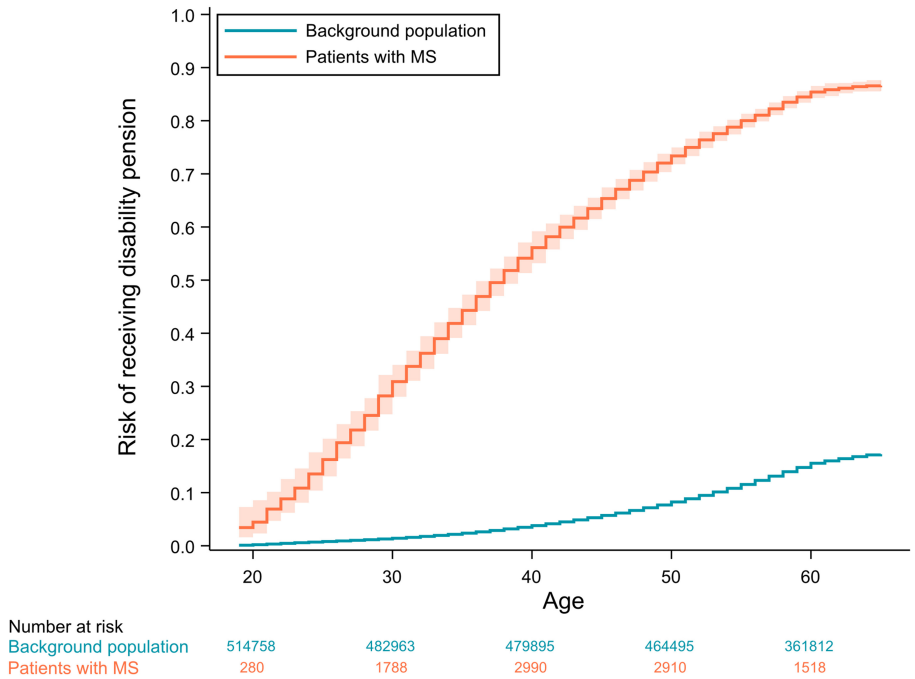

FIGURE 3 | Cumulative incidence curves of receiving disability pension with 95% confidence intervals adjusted for competing risks (age ≥ 65, emigration or death). Confidence intervals for the background population are not visible due to values very close to the incidence estimates.

This theory can also explain the relative preference of moderate effective DMTs at higher ages, which implies that emphasis is less upon treatment efficacy but rather tolerability in elderly patients. It is also worth to note that many of the patients in the highest age group were diagnosed around the time of the advent of disease modifying therapies. When the earliest drugs, interferon-βs, became available in Denmark in the late nineties, national treatment guidelines limited the immunomodulatory treatment to patients with two or more relapses per year. As such, only patients with high disease activity had access to treatment in those years.

When assessing the prevalence of no income from earnings in the cross-sectional analysis, we found a significantly increased prevalence for patients with MS across all age categories. This finding is like that of previous studies from Sweden showing a higher percentage with at least one record of no income from salaries within 10 years after diagnosis, and in general, a lower income after diagnosis compared with healthy controls (4, 29). The differences between patients and controls were considerably smaller than those seen for receiving disability pension, which indicate that income loss for 1 year is a more frequent occurrence in the background population, in turn increasing the validity of our application of a composite income–outcome in the longitudinal analysis. There is a surprisingly large number of events in both the MS and the control group among those aged 18–24 having no income, which is mainly due to students not having an income from earnings. The prevalence ratio should remain robust since education is free and with equal access in Denmark.

Looking at the cumulative incidence functions of the absolute risk of losing all income from earnings for 2 consecutive years, the risk is diverging until the control population reach their fifties and the risk starts converging, due to healthy controls also beginning to lose their income from earnings. Interestingly, the same age-related acceleration of income-loss is not seen for the patients with MS. This is possibly due to a selection of socioeconomically robust patients in the higher age groups that have managed to maintain an income up until this point. These patients, who have not lost their income this late in life, are likely systematically different from the rest of the patient population, in that they might be more resilient or have an income from earnings not as dependent on physical or mental ability.

When assessing the risk of disability pension, we found that the current Danish population of patients with MS of working age had a 22.8% higher prevalence (30.5 vs. 7.7%) of receiving disability pension compared with controls. These differences were statistically significant across all age categories except the youngest, probably due to low overall occurrence of the outcome in this category. The Social Pension Law of Denmark allows for granting of disability pensions at ages below 25, but only under extreme circumstances.

In the longitudinal analysis, we found a substantial difference in the HRs for receiving disability pension for all age groups. A peak in HR of disability pension was found at ages of 25–34 years. The HRs of income loss did not display the same rise and fall and remained stationary during midlife. The cumulative incidence curve clearly supports a massively increased absolute risk of receiving disability pension in patients with MS, with

patients showing a similar risk of receiving disability pension at the age of 25 as the controls do at the age of 65. The ever-increasing divergence of the risk is in line with other studies, which shows that the risk of losing employment is related to higher age but also increased disability, lower education, higher age at onset, longer disease duration, and more fatigue (30, 31).

Our MS population was nationwide and population-based. The analysis would be strengthened by the application of multivariate adjustment, specifically with the addition of EDSS scores; however, the untreated patients are not seen frequently in the clinic, and thus, many do not have recent EDSS scores available. Another limitation is the absence of descriptive socioeconomic variables such as level of education and type of labor on the current population with MS in Denmark. An addition of these would have made comparisons with countries of dissimilar structures of social legislation and health care easier. Citizens of Denmark are provided free education and free access to health care making the Danish population very homogenous. Thus, the comparisons within the Danish population in this study are only prone to small amounts of related confounding.

A strength of this study is the large amount of data we have in the DMSR linked to other population-based registries, which makes the results less prone to random variation, giving us the possibility to calculate estimates with high precision. Another strength is that independently collected data in large Danish registries and databases can be merged by the Danish unique personal identification code. Our data on income and disability pension have a virtually complete capture rate and it is representative of the whole Danish population due to the nationwide nature (14, 18).

Our results support the hypothesis that MS drastically worsens the socioeconomic status of patients. EDSS has been used as an outcome measure for decades, and it is easy to compare between patients cross-sectionally. However, the impact of disease experienced by the patients may raise other concerns such as fatigue, sleep, or their ability to maintain their job; important aspects of life are not captured by EDSS.

Socioeconomic status is not only dependent on income from earnings and disability pension. We hypothesize that our results may represent a tendency of MS influencing many socioeconomic factors of the patients, so that these trends could be shown for other aspects such as care and assistance at home or education after diagnosis, though more research is needed to reveal the exact nature of associations between MS and other socioeconomic factors. Using socioeconomic parameters as outcomes in MS research is warranted as these are highly affected by the disease while having substantial consequences for patients with MS on a personal level. Further, receiving disability pension or losing all yearly income constitute relevant, somewhat "hard" endpoints, that are likely very reflective of the functional capacity of patients. Such studies are well-suited and feasible to perform using Danish nationwide MS data allowing for linkage to registries holding socioeconomic data.

We found that the Danish patients with MS are at a higher risk of losing all income from earnings and at a much higher risk of receiving disability pension compared with healthy controls. Both risks were shown to increase drastically by age. Although our results focus on the ability of patients to maintain a job, we hypothesize that MS also influences many other socioeconomic factors in life.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MW-H, MA, and MB: design, conceptualization, methodology, software, data analysis, visualization, drafting, and revision for intellectual content. MM: design, conceptualization, methodology, drafting, revision for intellectual content, supervision, funding acquisition, and resources. All authors contributed to the article and approved the submitted version.

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Efficacy of Disease Modifying Therapies in Progressive MS and How Immune Senescence May Explain Their Failure

Navid Manouchehri[1], *Victor H. Salinas*[1], *Negar Rabi Yeganeh*[2], *David Pitt*[3], *Rehana Z. Hussain*[1] *and Olaf Stuve*[1,4]*

[1] Department of Neurology, The University of Texas Southwestern Medical Center, Dallas, TX, United States, [2] Department of Radiopharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran, [3] Department of Neurology, Yale University, New Haven, CT, United States, [4] Neurology Section, VA North Texas Health Care System, Medical Service Dallas, Veterans Affairs Medical Center, Dallas, TX, United States

The advent of disease modifying therapies (DMT) in the past two decades has been the cornerstone of successful clinical management of multiple sclerosis (MS). Despite the great strides made in reducing the relapse frequency and occurrence of new signal changes on neuroimaging in patients with relapsing remitting MS (RRMS) by approved DMT, it has been challenging to demonstrate their effectiveness in non-active secondary progressive MS (SPMS) and primary progressive MS (PPMS) disease phenotypes. The dichotomy of DMT effectiveness between RRMS and progressive MS informs on distinct pathogeneses of the different MS phenotypes. Conversely, factors that render patients with progressive MS resistant to therapy are not understood. Thus far, age has emerged as the main correlate of the transition from RRMS to SPMS. Whether it is aging and age-related factors or the underlying immune senescence that qualitatively alter immune responses as the disease transitions to SPMS, that diminish DMT effectiveness, or both, is currently not known. Here, we will discuss the role of immune senescence on different arms of the immune system, and how it may explain relative DMT resistance.

Keywords: adaptive immunity, innate immunity, multiple sclerosis, immunosenescence, progressive multiple sclerosis, disease modifying therapies

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***Correspondence:**
Olaf Stuve
olaf.stuve@utsouthwestern.edu

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INTRODUCTION

Multiple sclerosis (MS) is the most prevalent inflammatory disorder of the central nervous system (CNS) with a presumed autoimmune pathogenesis. MS was traditionally viewed as a T cell-mediated inflammatory disorder based on numerous observations made over the span of many decades. Aside from the abundance of lymphocytic infiltrates in MS lesion biopsies, other factors included: (A) the induction of the experimental autoimmune encephalomyelitis (EAE) model of MS in healthy recipient animals by adoptive transfer of myelin-reactive $CD4^+$ T helper (Th) cells from previously immunized donor mice (1); (B) the genetic association of MS with human leukocyte antigen (HLA) DRB1*15:01 (2), a major histocompatibility complex (MHC) class II molecule, required for the presentation of linearized peptides to $CD4^+$ Th cells; (C) the failed attempt to treat MS patients with an altered peptide ligand of myelin basic protein (MBP) that activated MBP-reactive $CD4^+$ Th cells, leading to disease exacerbation instead (3); (D) the initiation

and re-activation of MS with immune checkpoint inhibitors during cancer therapy (4); and (E) the beneficial effects of pharmacotherapies in early relapsing MS that deplete T cells, or sequester them out of the CNS (5, 6). This last aspect has illustrated how in early MS, relapses and new MS brain lesions are triggered and perpetuated by T cells and possibly other bone marrow-derived immune cells (7). The success of B cell depleting therapies in treating active MS, further corroborates the role of bone marrow-derived immune cells outside of the T cell compartment in pathogenesis of the disease (8–10). Changes in the clinical phenotype of MS, including treatment responsiveness will likely be linked to these cells as well.

A clinical course typified by relapses followed by periods of remission defines relapsing-remitting MS (RRMS) (11, 12). Patients with early MS who display clinical and paraclinical magnetic resonance imaging (MRI) disease activity gain a detectable and substantial benefit from receiving disease modifying therapies (DMT); patients without these evidences of disease activity, are defined as progressive MS (PMS); specifically, based on the 2013 Lublin criteria, PMS patients accrue objectively documented neurological disability without intermittent recovery and do not appear to receive any benefit from DMT (12, 13). Thus, the molecular and cellular signature of MS, as the primary therapeutic targets, change with age and disease becomes non-active. PMS at this stage is considered either secondary MS (SPMS) when following a period of RRMS, or primary progressive MS (PPMS) in lieu of relapsing disease activity (11, 12). PPMS patients are ∼10 years older upon diagnosis than RRMS patients. A subsection of patients with PMS, do show disease activity as defined above (12). There is no disease biomarker to indicate when the transition from RRMS to SPMS starts or is completed.

Currently, different hypotheses try to explain this transition; to date, age has been the most relevant prognostic factor underlying the transition from active RRMS to non-active SPMS (14–17). In contrast, a meta-analysis of all blinded, randomized clinical trials of DMT for RRMS indicated that DMT efficacy were independent of the recipients' age (18) despite a clear trend toward reduced effectiveness. Unfortunately, individualized data was not made available to the authors, and these results have to be interpreted with some caution.

As a biological correlate to age, immunosenescence has been advocated as a candidate to explain diminished DMT efficacy in PMS (19). Immunosenescence correlates with age relative to overall life expectancy (20). It is often accompanied by a decline in key immune functions such as the capacity for strictly non-self-antigen presentation and breadth of antigen recognition, the formation of long-lasting immune memory, and active immune surveillance (20). Here, we discuss whether immunosenescence contributes to the transition from active to non-active MS and how that correlates to loss of DMT efficacy in the context of PMS.

AGE, IMMUNOSENESCENCE, AND LOSS OF DMT EFFICACY

The transition to SPMS may not be entirely influenced by age. It takes place on average two decades following the clinical diagnosis of RRMS in adult-onset MS (AOMS) (21). Given that currently approved DMT remain effective even in patients with late-onset MS (LOMS), diagnosed in 50-year-old patients or older, it appears counterintuitive to attribute DMT unresponsiveness to age alone. Although, PPMS is more prevalent among LOMS than AOMS, still, nearly 50% of LOMS cases are RRMS and respond to DMT (18, 22). Immunosenescence as a potential contributor to DMT-resistance may be present in both AOMS and LOMS, and not entirely driven by age.

A correlate of intact adaptive immune function is responsiveness to vaccination with neo-antigens. Expectedly, vaccine efficacy wanes in elder populations (23); however, data on vaccine response among elderly RRMS demonstrate substantial adaptive immune response, despite long term DMT treatment with proven negative effects on vaccine response (24), reiterating how age alone does not define quality of immune response.

Current DMT in MS, including interferons (25–27), copolymers (28) depleting agents against CD20 (8–10) and CD52 (29), nucleoside synthesis antagonists (30–32), sphingosine-1 phosphate receptor modulators (33–36), nuclear 1 factor (erythroid-derived 2)–like 2 modulators (37) or α4-integrin antagonists (38, 39), aim to either deplete lymphocytes, modulate pro-inflammatory features or inhibit their traffic into the CNS. These DMT classes are approved for use in active MS. The validating trials for the only two FDA-approved DMT for use in SPMS, namely, cladribine (40, 41) and siponimod (34) recruited a mix of active and non-active progressive MS patients, limiting their relevance in pure non-active MS cohorts (13). These trials likely benefited participants with residual active MS (42). Conceivably, immunosenescence, both predates and promotes the transition from DMT-responsive active MS to DMT-resistant non-active MS. However, instigators or accelerators of immunosenescence in the context of MS require further elucidation.

ADAPTIVE IMMUNOSENESCENCE AND DMT-RESISTANT PMS

The adaptive immune system is not fully competent at birth; it becomes fully functional post-puberty and in early adulthood, declining progressively thereafter (43–46). Despite the age-associated decline in thymic epithelial tissue, it has been demonstrated that both the thymic cortex and thymic medulla function throughout life (47–49); however, the inevitable thymic involution is accompanied by the reduction of T cell diversity (49). Intact and functional thymic epithelium continually produces T cells migrating out of thymic medulla to peripheral lymphoid organs (50, 51). T cells generated from thymopoiesis have a full T cell receptor (TCR) repertoire, and are capable of generating responses to neo-antigens. In contrast, expansion of the peripheral T cells, driven by thymic involution may lead to repertoires limited to those of existing memory T cells and reduced capacity of immune response to new antigens (52–55). As mentioned, T cells are critical in initiating and perpetuating inflammation in active MS (7). MS pathogenesis potentiates T cell-antigenic spreading and repeatedly stimulates CNS-specific

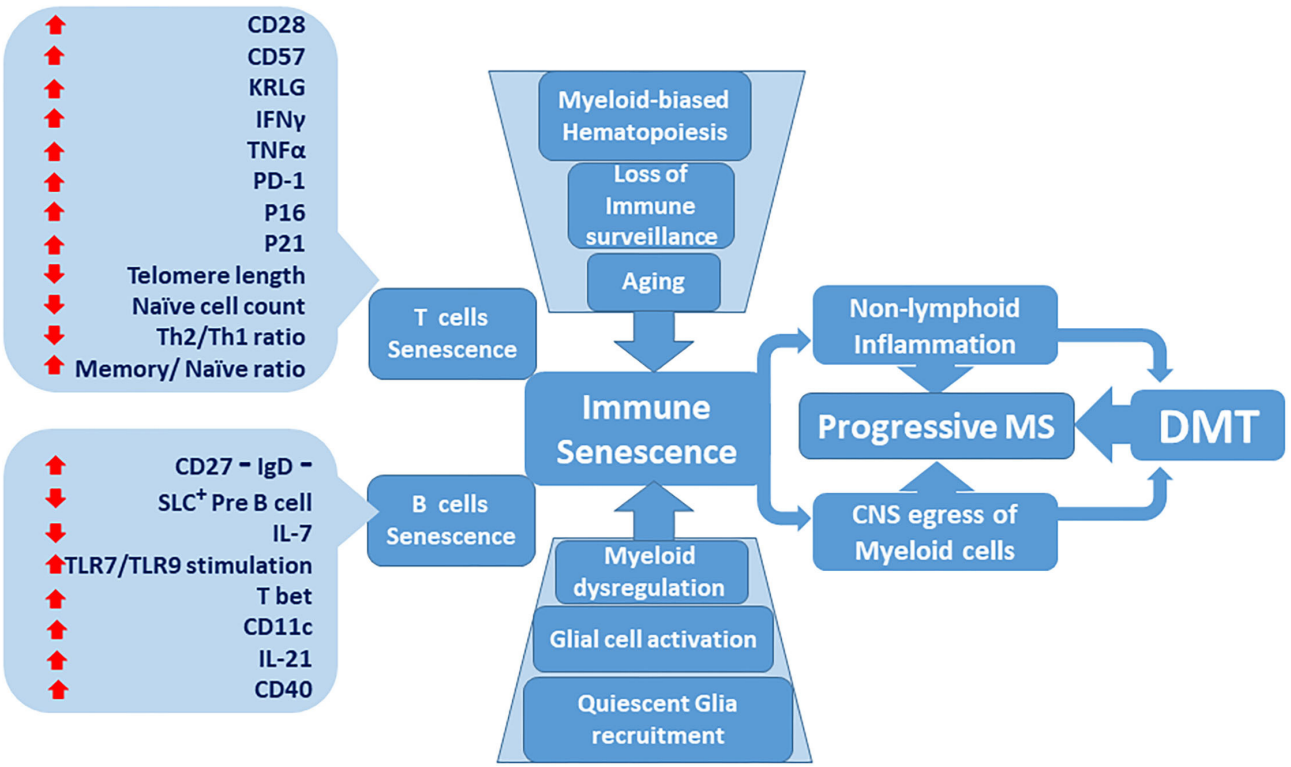

FIGURE 1 | Immune senescence contribution to the lack of effectiveness of disease-modifying therapies (DMT) in progressive multiple sclerosis (MS). Senescent T and B immune cell phenotypes become more predominant as the immune system ages. Age-dependent deterioration of tissues including central nervous system (CNS) glial cells increases. Bone marrow-derived myeloid cells are activated and compound adaptive immune senescence. Dysregulated myeloid cells prospectively accumulate within the CNS milieu. Inflammatory MS, characterized by active relapses, transitions to non-active progressive MS, which is resistant to DMT.

T cells (56). Suppressed CD28 expression, mediated by repeated antigenic stimulation is associated with senescent phenotypes in T cells (**Figure 1**) (23, 57–61). A similar phenotype of $CD28^{low}$ T cells is detectable in the pool of circulatory effector memory T cells with senescent attributes in the context of MS. (62, 63). Immunosenescence is not restricted to cellular immunity and similarly carries over to B cell-mediated immune responses (61). Noticeably, non-cellular adaptive immune responses mediated by B cells have been implicated by preclinical models as mediators of CNS autoimmunity (64). The Epstein-Barr virus (EBV), a plausible pathogenic MS trigger, is in fact a B cell tropic infection (65). EBV infection primes polyclonal populations of B cells to avidly present self-antigens to autoreactive $CD8^+$ cytotoxic T cells (66). Post-mortem studies that have yet to be reproduced showed abundant EBV infected B cells within actively demyelinating MS lesions (66, 67). Similar to T cells, constant B cell activation may drive premature senescence (68). Interestingly, surface expression of CD40, a correlate of B cells antigen presentation and memory formation, signifies senescence and is elevated in EBV infected B cells (69). In B cell senescence, it is quality rather than quantity of humoral response that declines, resulting in comparable volume of antibodies, albeit less effective. This is evident from diminished antibody specificity for foreign antigens, and decreased predominance of IgG isotypes along with lowered affinity of antibodies (70). Prematurely senescent B cells provide antigen presentation for expansion and maintenance of T cells in autoimmune disease like MS. Namely, increased signaling via CD80, CD86, CD11c, and CD40 by B cells in MS patients is higher than healthy controls and responsible for promotion of inflammatory T cell responses. Ultimately, the expression of these markers correlates with exhausted or senescent B cell phenotypes (**Figure 1**) (23, 57–61, 71). As discussed earlier, anti-CD20 agents that deplete B cells have become a mainstay of therapy for active MS with proven efficacy (72). However, B cell depletion with the humanized anti-CD20 monoclonal antibody (72) ocrelizumab, which is approved for PPMS, does not deliver significant neuroprotective effects as assessed by serial blood measurements of neurofilament light chain (NfL) (73). $CD20^+$ cells are not a single population and range from naïve B cells to fully matured memory cells. Since production of new pro-B cells is directly affected by senescence (74), continued treatment with anti-CD20 therapies likely depletes naïve B cells with diverse B cell receptor (BCR) repertoires. Likewise, continuation of T cell-depleting DMT in non-active PMS will likely result in T cells that lack diverse naïve cells. In this manner, increasing age and DMT therapy compound adaptive immunosenescence in MS. Nevertheless, if adaptive immunity was relevant in perpetuating disease progression in

PMS, DMT effectiveness should have remained constant or even slightly improved since PMS is dominated by senescence. In fact, if DMT unresponsiveness in PMS was driven predominantly by adaptive immunity, decreased potency of senescent adaptive immunity would have led to improvement of clinical outcomes in elder patients. Since DMT optimally address adaptive immune cells in the periphery, DMT resistance in non-active PMS requires explanations beyond adaptive immune components or peripheral compartment.

INNATE IMMUNOSENESCENCE AND DMT RESISTANCE IN PMS

Innate immunity is influenced by the immunosenescence. Contrary to adaptive immunity, the innate immune system in mammals is considered functional at birth, and retains most of its function throughout life (61). Cell migration, adhesion and phagocytosis of polymorphonuclear leukocytes (PMN) were believed to stay virtually unaltered by aging (75–78); however studies have shown that certain innate immune cell functions may falter with age (79–81). Immunosenescence pushes innate immune cells toward functional dysregulation that compounds the effects of suboptimal senescent adaptive immunity. In MS, effects of immunosenescence on innate immune are noticeable in four domains; namely, (1) Aged innate immune system manifests with the preponderance of dys-homeostatic phenotypes and *forme pleine* of the ideally self-limiting responses. This is likely to promote chronic and continued tissue destruction, sub-functional remodeling and delayed healing (81–84). (2) Within the CNS, myeloid cells, as antigen presenting cells (APC), re-activate and retain $CD4^+$ T cells, and contribute to effective immune surveillance (85). These cells are altered via senescence. There are three compartments within the brain where myeloid cells exert their effect: (A) the parenchyma, (B) cerebral perivascular spaces (CPVS) and (C) meninges. Parenchymal microglia are tissue-intrinsic macrophages of the CNS (86–88). The other relevant compartments for antigen presentation are CPVS and meninges, which are populated by monocyte-derived macrophages and dendritic cells (DC). In CX_3CR_1 GFP^+ mice, it was demonstrated that microglia and monocyte-derived brain macrophages are distinct entities (89, 90). Meninges have been implicated as an anatomic site in host defense and autoimmunity. Major histocompatibility complex II (MHCII)-positive cells, detectable within all meningeal layers (91), include monocyte-derived macrophages, monocyte-derived DC (mDC) and classical DC (cDC) (92). While some studies reported DC from young and aged humans having similar surface expression of MHCII molecules and elicited equal T cell proliferation, other investigators demonstrated significantly lower MHCII expression by DC in the elderly (93, 94). (3) During hematopoiesis, immunosenescence reduces lymphopoiesis in favor of heightened myelopoiesis, resulting in a net increase in myeloid cell output (59). (4) Inflammaging, defined as chronic, low grade and sterile inflammation, despite the overall diminishment in immune functions, increases with age (81, 95). Bone marrow-derived myeloid cells (BMC), activate and upregulate surface adhesion molecules in response to inflammation (96). This may allow BMC to penetrate and accrue within target tissues such as CNS (97).

CNS microenvironment in response to the aforementioned events may shift to a dys-homeostatic state adopted by CNS-resident myeloid cells (97). Previous observations postulated that compared to naïve quiescent microglia, activated microglia and infiltrated BMC during CNS inflammation upregulate inflammation-associated signals (92). Myeloid cells within the CPVS are constantly replaced by BMC, and this turnover is accelerated during inflammation (98). During EAE, parenchymal microglia and BMC exhibited mutual activation markers following the onset of clinical disease in mice. Specifically, clinical disease onset was temporally associated with the appearance of BMC in the CNS inflammatory milieu (97). This was not a transient event and the newly present activated BMC were likely retained within the CNS microenvironment and merged with the activated microglial pool (97). It is still unclear to what extent these changes advance functional disarray and homeostatic disturbance; however, current DMT in MS do not primarily target BMC. Certain DMT might modulate the trafficking of BMC into the CNS, as demonstrated with natalizumab therapy (99). However, the ASCEND trial, a phase 3 study on the efficacy of natalizumab therapy in SPMS, failed to show meaningful clinical benefits (100), perhaps suggesting that ongoing migration of BMC in SPMS is no longer highly relevant to disease progression.

"Smoldering" MS lesions are a candidate to explain PMS and chronic destruction of CNS parenchyma without evident inflammation. They are dominated by the presence of perilesional activated myeloid cells without lymphoid inflammation (101, 102). Whether these myeloid cells are CNS intrinsic or bone marrow-derived, or a mixture of both is incompletely understood. Smoldering MS is more prevalent among SPMS patients who are on average older than active MS patients, however, as we discussed before, age likely is not the driving factor. The transition to SPMS takes place faster in LOMS patients; however, accrual of disability for LOMS patients during years living with SPMS is slower in comparison (21). Possibly, later onset and relatively brief inflammatory phase in LOMS, spares them from fully-recognized disease burden. In the context of non-active PMS, antigen-independent and compartmentalized chronic inflammation, shielded from therapeutic efforts, is plausible. The corollary to this hypothesis would be that even outside of common DMT, other anti-proliferative therapies might fail to provide meaningful clinical benefit to PMS (103), a logic that should guide hematopoietic stem cell transplants as well (104). Furthermore, if myeloid cells drive PMS, they probably do so in a stage-specific fashion; (1) BMC likely enter the CNS throughout active and non-active MS and are retained within the CNS; and (2) altered CNS-intrinsic myeloid cells, respond to BMC presence, at sites similar to the border of smoldering lesions. Adaptive immune cells most likely instigate these events at first; during non-active MS, such external cues may no longer be absolutely required and suppression of adaptive immune system *via* DMT, thereafter provide little clinical benefit.

GLIAL SENESCENCE AND DMT RESISTANCE IN PMS

Glial cells including astrocytes and microglia are critical in CNS. Immune functions related to astrocytes in the context of MS and its preclinical models have recently attracted renewed interest; however their pathogenic role in MS is currently less clear. For instance, the depletion of astrocytes worsens clinical disease in an acute EAE model but ameliorates progressive EAE (105). A subpopulation of astrocytes expresses complement component 3 (C3) in response to interleukin-1 alpha (IL-1α) tumor necrosis factor alpha (TNFα) and complement component 1, subcomponent q (C1q), and possesses neurotoxic properties and are upregulated in MS lesions (106). Their development underscores a bi-directional interaction between astrocytes and myeloid cells. Pro-inflammatory signals from Microglia and BMC in CNS are a primary inducer of such astrocytes while activated astrocytes allow further recruitment and entry of pro-inflammatory monocytes to the CNS; this multiplicative effect may have been intercepted by astrocyte depletion leading to the aforementioned amelioration of chronic EAE (106, 107). Furthermore, senescent astrocytes, may contribute to neurodegeneration in an overburdened neural network post demyelination. Astrocytes possess star-shaped appearance, and are intimately associated with the CNS vasculature (108). Together with endothelial cells and pericytes, astrocytes are critical in forming and maintaining the blood brain barrier (BBB). Astrocytes can express MHC class II molecules in defined experimental conditions which endows them to serve as potential APC to $CD4^+$ T helper cells (109–111). Cellular changes associated with astrocyte senescence include the increased expression of glial fibrillary acidic protein (GFAP) and vimentin (112, 113). This is at least partly driven by increased signaling of transforming growth factor beta 1 (TGFβ1). TGFβ1 inhibits astrocyte proliferation and induces a senescence-associated secretory phenotype (SASP), which involves an enhanced expression of inflammatory molecules (19, 114).

Inflammation induced phenotypes in microglia also mimic senescence. Specifically, CNS microglia exhibit a phenotypical profile that has been frequently associated with aging in the context of neurodegenerative disorders (115). These attributes, including increased iron storage, production of pro-inflammatory cytokines, lower motility and diminished phagocytic capacity are not strictly age-dependent and are inducible by other insults as well. Single cell transcriptomic studies on EAE models as well as human samples have shown presence of distinct microglia-like cells in CNS inflammation (97, 116). Microglia are maintained by local proliferative self-renewal (90). Within the CNS, functional properties of microglia, as tissue resident myeloid cells, across age groups are likely constant. Interestingly, it was recently shown that microglial density in the brain increases in aged mice (117). However, the authors had utilized markers that correlated with activated microglia, namely Iba-1. Therefore, the observed increase in microglia density with age might point more toward heightened microglial activation and subsequent reduction in the pool of homeostatic quiescent microglia. Senescent microglia are found within the brains of PMS patients despite pronounced reduction in inactive lesions (118, 119). As discussed, these cells might exert their role at the border of smoldering lesions (102). Phagocytosis of myelin debris supports re-myelination efforts; microglial depletion associates with loss of phagocytic capacity in the microenvironment of MS lesions, likely promoting dysmyelination. Aged human microglia exhibit proclivity to express ferritin, believed to be associated with senescence (120). Increased iron uptake likely follows the destruction of iron-containing oligodendrocytes in MS and is observable in aged microglia. These limitations to CNS re-myelination efforts, possibly lend to PMS phenotype.

Glial cells are not a primary target for current DMT, and their role in relation to why DMT fail remains to be explained. The restricted CNS bioavailability of most DMT, is unlikely to impact glial cells. Further studies are required to elucidate the significance of these observations and their potential for development of novel therapeutics.

TOWARD A THEORY FOR DMT RESISTANCE IN PROGRESSIVE MS

Based on extensive data from clinical trials and post-approval observational studies it is evident that the current therapeutic dogma in MS, namely the depletion of inflammatory adaptive immune cells or their sequestration out of the CNS is effective during active RRMS. Current data gleaned from clinical trials suggest that most DMT have minimal effects on non-active progressive MS without signs of disease activity (13, 34, 73, 121, 122); therefore administration of DMT that target immune components may not be in the best interest of these patients. Alternatively future therapeutic endeavors may benefit from incorporating strategies that cover innate immunity and glial targets within the CNS.

The evidence presented here supports a view of DMT resistance associated with immunosenescence in PMS (**Figure 1**). Possible pharmacological efforts to address immunosenescence may adopt designs that identify senescence-specific factors, amenable to modulation. A desired goal and measure of success could be delayed transition to non-active PMS. Current DMT, as effective as they are in controlling RRMS, are unqualified to address the breadth of ongoing deleterious effects of MS (123–125), including the role of innate immune cells and CNS glial components. Myeloid cells, as potential candidates for targeted novel therapies in non-active PMS, retain phenotypical plasticity. Curtailing the dys-homeosatic signaling in these cells and safeguarding against further disruption of CNS quiescence, is a biological plausibility (126, 127). Given the current availability of analytic tools with single-cell resolution, deep characterization of myeloid sub populations and definition of exclusive phenotypical signatures in pertinent compartments is both feasible and indispensable. Molecular targets, acquired through this process could serve as a map for pre-clinical efforts (128, 129).

In conclusion, immunosenescence as a multivariate phenomenon, is not defined by advancing age alone and its

constellation of immunological effects over time culminates to DMT resistance in PMS. Current DMT mechanisms of action are optimized to mitigate inflammation-induced damage during active MS. They are limited to address the inevitable transition to non-active PMS or remain effective thereafter. Clinical and biological data call for a more targeted approach with an emphasis on myeloid cells, innate immunity components and glial cells in the future.

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NM and NR surveyed the literature. NM, VS, and NR drafted the manuscript. RH, DP, and OS provided expert opinion and edited the final manuscript. All authors contributed to the article and approved the submitted version.

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