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Early predictive risk factors for dimethyl fumarate-associated lymphopenia in patients with multiple sclerosis

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ABSTRACT

Background: Lymphopenia is a major concern in MS patients treated with dimethyl-fumarate (DMF) as it increases the risk of progressive multifocal leukoencephalopathy. A pronounced reduction in absolute lymphocyte counts (ALCs) early after treatment initiation has been suggested to be associated with the occurrence of lymphopenia thereafter.

Objectives: To identify risk factors for DMF-induced lymphopenia and evaluate whether the degree of decrease in the ALCs three months after initiation of DMF treatment is a predictor of the subsequent development of lymphopenia.

Methods: In this real-world Spanish prospective multicenter study conducted in MS patients who started DMF between 2014 and 2019, we analyzed the association between DMF-related lymphopenia and the percentage of early ALCs decline using regression models, considering both, significant lymphopenia (grades 2 + 3) and severe lymphopenia (grade 3). The cutoff values of early ALCs declines were obtained using the ROC curve.

Results: Among 532 MS patients treated with DMF, 193 (36.3%) developed any grade of lymphopenia. Older age and lower ALCs at treatment onset predicted the risk for lymphopenia but the best predictive risk factor was the reduction of ALCs within the three first months of treatment. Specifically, a reduction in ALCs≥21.2% was associated with a 6.5-fold higher risk of developing significant lymphopenia, and a decrease in ALCs≥40.2% with a 12.7-fold higher risk of developing severe lymphopenia.

Conclusions: A pronounced reduction in ALCs early after initiation of DMF in MS patients is the best predictive risk factor for the subsequent development of significant lymphopenia.

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1. Introduction

Delayed-release dimethyl fumarate (DMF; Tecfidera, Biogen, Weston, MA) is an oral disease-modifying therapy (DMT) approved for the treatment of adult patients with relapsing multiple sclerosis (RMS) (Tecfidera, 2014). During the first year of DMF treatment, lymphocyte counts decrease by approximately 30% and then stabilize (Gold et al., 2012; Fox et al., 2012, 2016). Although no increased incidence of serious infections was observed in patients with DMF-associated lymphopenia in clinical trials and their 9-year extension study (Gold et al., 2020), rare cases of progressive multifocal leukoencephalopathy (PML) have occurred in the postmarketing setting (Jordan et al., 2020). Thus, it is particularly important to know which patients are at higher risk for developing grade 2 and 3 lymphopenia, in order to make therapeutic decisions and carefully balance the risk-benefit of early treatment discontinuation (Longbrake et al., 2015).

We previously reported that a pronounced reduction in absolute lymphocyte counts (ALC) three months after initiation of DMF treatment was associated with subsequent development of lymphopenia in 106 RMS patients (Sainz de la Maza et al., 2019). Given that this could be a useful and accessible tool in clinical practice for early identifying of patients at increased risk of lymphopenia, we aimed to validate this data in an independent and larger cohort. We also analyzed the potential association between DMF-related lymphopenia and infections.

2. Methods

2.1. Study design, standard protocol approvals and patient consents

This work is a sub-study of an independent, multicenter, non-interventional, prospective real-world study conducted in 886 RMS patients from sixteen hospitals belonging to the Spanish Public Health System, fifteen in the Community of Madrid (central region) and one in Almeria (southern region) (Pilo de la Fuente et al., 2020; Sabin et al., 2020). Among them, ten hospitals in Madrid and one from Almeria participated in this research. The study was conducted according to requirements expressed in the Helsinki Declaration (Fortaleza, October 2013) and current Spanish legislation on observational studies (Ministerial Order SAS/3470/2009) and protection of personal data (Organic Law 3/2018, 5th December). Approval was obtained from the Ethical Committee of Hospital Universitario de Getafe (Madrid, Spain), the coordinator site of the study. All patients provided written informed consent before inclusion.

2.2. Study population

From February 2014 to May 2019, patients diagnosed with RMS according to the 2010 Revised McDonald Criteria (Polman et al., 2011) who received DMF and had available data on ALC were included in the study. The decision to prescribe DMF was made by treating physicians on an individual basis, in accordance with Spanish Society of Neurology clinical practice guidelines (García Merino et al., 2017). By protocol, only those patients who fulfill these laboratory criteria were included in this sub-study: (1) have available data on baseline ALC, and (2) have at least two consecutive ALC determination during the follow-up period.

2.3. Data acquisition and definitions

At baseline, demographic features, disease data (time since first symptoms, relapses in the past 12 months, previous history of DMT use), disability according to the Expanded Disability Status Scale (EDSS) score, radiological characteristics on magnetic resonance imaging (MRI) (number of T2 lesions and number of T1 gadolinium-enhancing lesions) and serial ALC determination were recorded.

Patients were evaluated every 6 months in order to assess both the occurrence of relapses and the EDSS score. MRI scans were performed yearly after DMF initiation. ALCs were recorded before and 3 months after DMF onset and every 3–6 months until discontinuation or up to 24 months in patients ongoing with DMF. When DMF was discontinued due to lymphopenia, ALCs were further monitored until full recovery. Lymphopenia was defined when a ALC value below lower limit of normality (LLN) was subsequently confirmed by another ALC determination. Taking the worst post-baseline ALC value, lymphopenia was classified according to the Common Terminology Criteria for Adverse Events (CTCAE v5.0) as follows: grade 1 (ALC 800-999/μl), grade 2 (ALC 500–799/μl) and grade 3 (ALC <500/μl) (National Cancer Institute, 2017).

Self-reported and/or laboratory data confirming infections that required medical attention were collected by the treating physicians. For this purpose, local researchers were periodically contacted by two investigator team members (JS, SSM) to monitor, collect and evaluate specific details on infections. The quality and homogeneity of the data was evaluated, periodically and independently, by three investigators of the coordinator team (YA, JS, SSM). Any inconsistency and/or erroneous data was sent back to the local investigator for correction.

2.4. Statistical analysis

Analyses were performed using the Statistical Package for Social Sciences, version 22.0 (IBM SPSS, Inc., Chicago, IL, USA).

For the purpose of the study, population was first divided into two groups: patients who developed any grade of lymphopenia during the follow-up time, and patients who did not developed lymphopenia. Since DMF-associated PML has been described mainly in patients with ALC < 800 (Jordan et al., 2020), we further studied the group of patients with grades 2–3 lymphopenia (hereinafter called "significant lymphopenia") and the group of patients with grade 3 lymphopenia (hereinafter called "severe lymphopenia"). To compared groups, $\chi 2$, Student's T or Mann–Whitney U were used when appropriate.

The primary endpoint was to assess whether there is an early ALC reduction cut-off associated with subsequent lymphopenia. The optimal cut-off of reduction in ALC within 3 months on DMF associated subsequent lymphopenia was established using a ROC curve.

The proportion of patients who developed lymphopenia of any grade, significant lymphopenia and severe lymphopenia was estimated using the Kaplan–Meier method.

A Cox regression model was carried out to evaluate the effect of clinical and analytical baseline characteristic in the risk for developing any grade of lymphopenia, significant lymphopenia and severe lymphopenia. Sex, age, clinical and radiological characteristics, baseline ALC, percentage change in ALC within three months of DMF treatment and previous immunosuppressive therapies (i.e., fingolimod, azathioprine, mitoxantrone, cyclophosphamide, natalizumab, alemtuzumab, ocrelizumab and rituximab), were considered in the model.

3. Results

3.1. Patients

Overall, 886 patients with RRMS initiated DMF from February 2014 to May 2019, but only those 532 who had data available on ALCs and fulfilled inclusion study criteria were included in this study (Fig. 1). Baseline demographic and clinical characteristics are shown in Table 1. Patients were 14 to 69 years old and had suffered their first relapse between 44 days and 35 years before DMF initiation. Four hundred and three (75.6%) patients had previously received another DMT. The mean±SD duration of DMF treatment was 3.13 ± 1.35 years.

3.2. Incidence of lymphopenia

A total of 193 (36.3%) patients developed any grade of lymphopenia. Considering worst post-baseline ALCs, the incidence of grade 1, 2 and 3

lymphopenia was 9.8% (52/532), 20.3% (108/532), and 6.2% (33/532), respectively. Patients who experienced any grade of lymphopenia were older at DMF initiation (44.0 vs. 38.9 years; $p < 0.0001$), had a longer disease duration (10.3vs. 8.5 years; $p = 0.005$), were more disabled (EDSS at baseline 2.31 vs. 1.71; $p = 0.0001$) and had lower ALCs prior to treatment (1805vs. 2192 cells/μl; $p < 0.0001$). There were no significant differences in number of relapses in the previous 12 months, radiological findings on MRI or previous DMT received between both groups. We further studied the differences in baseline demographic and clinical characteristics between patients with significant lymphopenia and patients without significant lymphopenia (i.e., grades 2–3 Vs. grades 0–1), and between patients with severe lymphopenia and patients without significant lymphopenia (i.e., grade 3 Vs. grades 0-1-2). Similar results were obtained (Table 1).

3.3. ALCs evolution during DMF treatment

Mean ALCs decreased by approximately 31.5% during the first two years of treatment within the overall population, remaining above LLN throughout the observation period. Time to appearance of lymphopenia ranged between 3 and 18 months (median 6 months). Survival curve of time to lymphopenia is represented in Fig. 2. Patients who subsequently developed lymphopenia experienced a higher decline in mean ALCs at three months of treatment than those who did not develop lymphopenia (34.5% vs. 9.8% decrease, $p < 0.0001$; Fig. 3). A 21.2% reduction in ALC three months after DMF onset was the optimal cut-off point associated with the subsequent development of any grade of lymphopenia (sensitivity 71.8%, 95% CI: 64.8–77.9; specificity 67.1%, 95% CI: 61.7–72) and of significant lymphopenia (sensitivity 77.4%, 95% CI: 69.6–83.7; specificity 64.3%, 95% CI: 59.2–69). For the subsequent development of severe lymphopenia, the optimal cut-off in ALC reduction was of 40.2% (sensitivity 65.6%, 95% CI: 46.8–80.8; specificity 77.4%, 95% CI: 73.4–80.9).

3.4. Risk factors for DMF-associated lymphopenia

With regard to the overall population, the Cox regression model showed that older age at DMF initiation (adjusted HR 1.03, 95% CI: 1.02–1.05, $p < 0.001$), lower baseline ALCs (adjusted HR 0.998, 95% CI: 0.998–0.999, $p < 0.001$), and a decline in mean ALCs ≥21.2% at three months of treatment (adjusted HR 5.09, 95% CI: 3.67–7.04, $p < 0.001$), increased the risk of DMF-associated lymphopenia. Using grades 2–3 lymphopenia as the outcome of interest, we again found that older age at DMF initiation, lower baseline ALCs, and a decline in mean ALCs ≥21.2% at three months of treatment increased the risk of significant lymphopenia. Having being previously treated with immunosuppressive DMTs vs. being treated-naïve increased the risk of grade 2–3 lymphopenia but only in the univariant Cox regression analysis (HR 1.82, 95% CI: 1.06–3.14, $p = 0.031$) (Fig. 4a). In relation to severe lymphopenia, also older age at DMF initiation, lower baseline ALCs and, most importantly, a decline in mean ALCs higher than 40.2% at three months of treatment increased the risk of grade 3 lymphopenia (Fig. 4b).

3.5. Lymphopenia related infections

Eight infections in seven patients were reported throughout the observation period. Characteristics of the patients and the infections are shown in Table 2. Six patients had been lymphopenic before the infection occurred. Three out of seven patients required DMF discontinuation due to the infection. Of the 339 non-lymphopenic patients, one (0.29%) had an infection, while of the 193 lymphopenic patients, six (3.11%) had infections, this difference being statistically significant ($p = 0.01$). The patient without lymphopenia who developed an infection was a 28-year-old man who had started dimethyl fumarate one month before developing a bacterial pneumonia when his lymphocyte count was 1600 cells/μl. He received specific antibiotherapy and did not require hospitalization.

3.6. ALCs evolution after DMF cessation due to lymphopenia

Of 193 patients with ALC ≤ LLN while on DMF, 42 (22%) patients required treatment discontinuation due to significant lymphopenia. Of the 23 patients who had data available on lymphocyte reconstitution, 20 (87%) patients reached ALC ≥ 800/μl at any time after DMF discontinuation date. The median time to reach ALC ≥ 800/μl was 5 months (range 1–24 months). Three patients did not reach this ALC threshold before initiating next DMT.

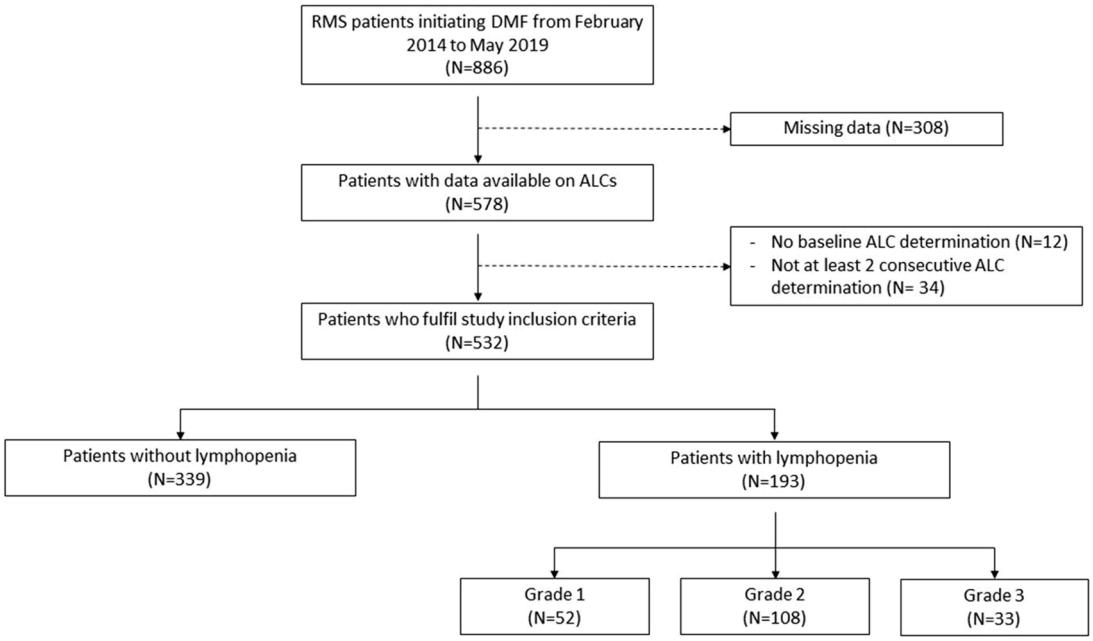

Fig. 1. Patient inclusion and stratification. Abbreviations: ALC, absolute lymphocyte count; DMF, dimethyl fumarate; RMS, relapsing multiple sclerosis.

4. Discussion

DMF has demonstrated efficacy and a positive risk-benefit balance in patients with RRMS in clinical trials (Gold et al., 2012, 2020; Fox et al., 2012, 2016) which has been confirmed in real-world studies (Pilo de la Fuente et al., 2020; Sabin et al., 2020; Mallucci et al., 2018; Granqvist et al., 2020). The most concerning adverse event of DMF is the decrease in ALC, as prolonged lymphopenia has been related with rare cases of PML (Jordan et al., 2020). Thus, the identification of patients at risk of lymphopenia is of high clinical interest. In this real-world study of DMF-associated lymphopenia, the largest reported so far, we studied 532 patients treated with DMF during a mean time of three years, 193 (36.3%) of whom developed lymphopenia. We evaluated whether the degree of decrease in the ALCs three months after initiation of DMF treatment was a predictor of the subsequent development of lymphopenia, as previously reported (Sainz de la Maza et al., 2019).

Different investigations explored risk factors for DMF-related lymphopenia. It has been widely described that older age at treatment initiation and lower baseline ALC are associated with higher incidence of severe prolonged lymphopenia (Gold et al., 2020; Jordan et al., 2020; Longbrake et al., 2015; Sainz de la Maza et al., 2019; Morales et al., 2020), as we have seen. However, literature on models to predict risk factors for DMF-related lymphopenia is scarce. We created a risk prediction model taking into account these well-known factors, and also the drop in ALC at three months on DMF. We found that older age and lower ALC at treatment initiation predicted the risk for significant lymphopenia but the best predictive risk factor was the early reduction of ALC. Specifically, a reduction in ALC $\geq$ 21.2% was associated with a 6.5-fold higher risk of developing significant lymphopenia, and a decrease in ALC $\geq$ 40.2% with a 12.7-fold higher risk of developing severe lymphopenia, confirming our previously reported findings (Sainz de la Maza et al., 2019). The negative predictive value was high for both cut-off points (88.3% for significant lymphopenia and 97.2% for severe lymphopenia, respectively), indicating an important value as a screening method. On that basis, patients not experiencing a rapid decrease in ALCs during first three months of treatment are unlikely to developed a significant or severe lymphopenia thereafter. Since, hypothetically, previous and recent exposure to immunosuppressive therapies may imply an increased risk of lymphopenia, we explored this factor in our risk prediction model and observed an increased risk of significant lymphopenia in the univariate analysis that was lost in the multivariate analysis. We did not observe any effect of previous immunosuppressive treatment in the risk of developing severe lymphopenia, most likely due to small sample size.

The 36% incidence of lymphopenia observed in our study is slightly lower than the 38–40% previously described in clinical trials and other real-world studies (Gold et al., 2020; Mallucci et al., 2018), probably due to different operational definitions of lymphopenia. In our study, a patient must have at least 2 ALC $\leq$ LLN at any time while on DMF to be considered as lymphopenic, since an isolated determination of ALC $\leq$ LLN should be a one-off event without clinically relevant implications. The percentage of grade 3 lymphopenia reported in the literature varies between 5.7 and 7% (Gold et al., 2012, 2020; Fox et al., 2012, 2016; Sainz de la Maza et al., 2019; Sabin et al., 2020; Mallucci et al., 2018) which is consistent with our results (6%).

The pattern of ALC decline is characteristic and highly constant in long-term studies (Fox et al., 2016; Gold et al., 2020; Sainz de la Maza et al., 2019; Mehta et al., 2019). It occurs within the first year and then stabilizes, as shown by our results. In our study, most cases of lymphopenia began to appear within first months of DMF treatment, and no cases of grade 2–3 lymphopenia emerged beyond the first year. In particular, we found the deepest decrease in ALC occurred within first three months of treatment, and was more pronounced in patients who subsequently developed lymphopenia.

The most concerning aspect of lymphopenia is the potential increased risk of infections, not only PML but also other serious infections. Most of the infections reported in clinical trials mainly comprised upper respiratory tract infections, urinary tract infections, and gastroenteritis (Gold et al., 2012; Fox et al., 2012). Severe infections were detected in 2% of the patients in all groups. In the 9 years' follow-up of the open extension of clinical trials, incidence of serious infections was similar across groups(Gold et al., 2020). When patients were stratified by on-treatment ALC, opportunistic infections as PML only occurred in patients with ALC<500/μl for $\geq$ 6 months (Mehta et al., 2019). It has been speculated that impaired lymphocyte function or reduction of CD8+ lymphocytes contribute to PML risk (Winkelmann et al., 2016). However, a real-word study of 72 RRMS patients treated with DMF suggests that peripheral blood lymphocyte changes are not associated with the rate of infections (Boffa et al., 2020). In our study, we have observed an association between having ALC $\leq$ LLN and experience an infection. However, it should be noted that surveillance for possible infections is greater and more active in lymphopenic

Table 1
Baseline characteristics according to the development of lymphopenia.

Variable	Total population (*n* = 532)	No lymphopenia (*n* = 339)	Grades 1–3 lymphopenia (*n* = 193)	Grades 2,3 lymphopenia (*n* = 141)	Grade 3 lymphopenia (*n* = 33)
Age, mean±SD (years)	40.8 ± 9.6	38.9 ± 9.5	44.0 ± 8.9****	45.0 ± 8.9****	46.6 ± 7.3***
Females, *n* (%)	372 (69.9)	232 (68.4)	140 (72.5)	106 (75.2)	25 (75.8)
Time since first MS symptoms, mean±SD (years)	9.17 ± 7.30	8.5 ± 7.1	10.3 ± 7.5**	10.5 ± 7.8*	11.5 ± 7.9
Previous DMT, *n* (%)					
None (treatment-naïve patients)	129 (24.2)	90 (26.5)	39 (20.2)	25 (17.7)	4 (12.1)
Immunomodulatory DMT	325 (61.1)	203 (59.9)	122 (63.2)	93 (66.0)	22 (66.7)
Immunosuppressive DMT	78 (14.7)	46 (13.6)	32 (16.6)	23 (16.3)	7 (21.2)
N. of relapses in the previous 12 months, mean±SD	0.67±0.72	0.71±0.73	0.61±0.69	0.60±0.70	0.73±0.91
EDSS score at baseline					
Mean±SD	1.93 ± 1.55	1.71 ± 1.42	2.31 ± 1.68***	2.41 ± 1.76***	2.81 ± 1.81**
Median [range]	1.5 [0–7.5]	1.5 [0–7]	2 [0–7.5]	2 [0–7.5]	2 [0–6.5]
N. of gadolinium-enhancing T1-weighted lesions, mean±SD	(*n* = 443) 0.79 ± 1.99	(*n* = 284) 0.90 ± 2.30	(*n* = 159) 0.61 ± 1.28	(*n* = 117) 0.60 ± 1.37	(*n* = 23) 0.48 ± 1.08
N. of hyperintense T2-weighted lesions	(*n* = 448)	(*n* = 286)	(*n* = 162)	(*n* = 119)	(*n* = 24)
$\leq$ 9, *n* (%)	70 (15.6)	50 (17.5)	20 (12.3)	17 (14.3)	3
> 9, *n* (%)	378 (84.4)	236 (82.5)	142 (87.7)	102 (85.7)	21
Baseline ACLs, mean±SD (cells/μl)	2052 ± 712	2192 ± 752	1805 ± 559****	1796 ± 570****	1608 ± 493***

ALCs: absolute lymphocyte counts; DMT: disease modifying therapy; MRI: magnetic resonance imaging; *n*: number; SD: standard deviation.
Comparison groups: Grade 0 Vs. Grades 1–3; Grades 0–1 Vs. Grades 2–3; Grades 0–2 Vs. Grade 3.
Significant differences between groups (U Mann–Whitney): *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

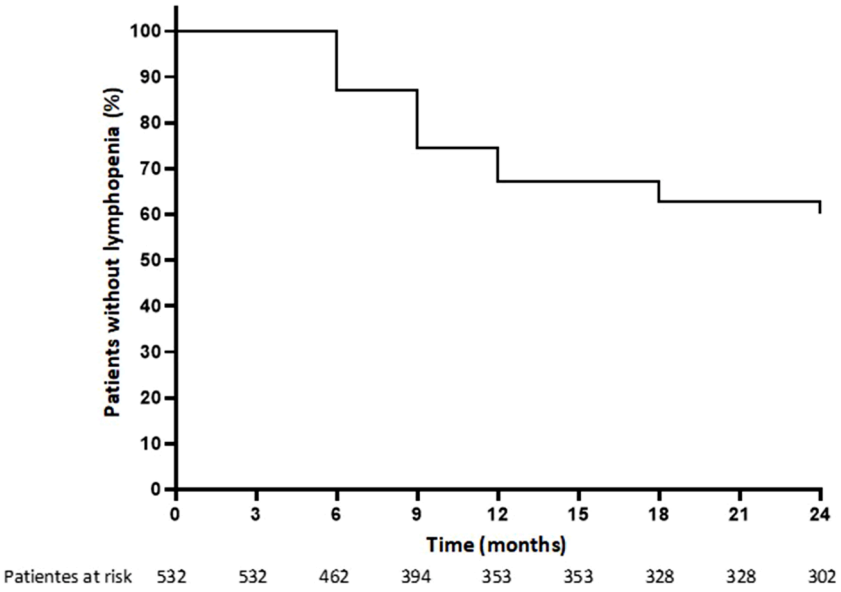

Fig. 2. Survival curve of time to appearance of lymphopenia.

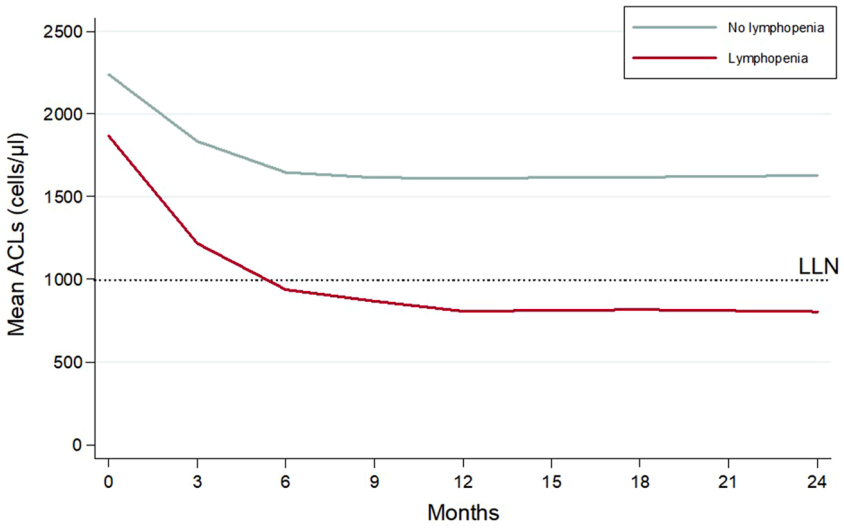

Fig. 3. Mean absolute lymphocyte count (ALC) changes along dimethyl fumarate treatment in patients who developed (red line) or not (green line) lymphopenia. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

patients. The most frequent infections were respiratory tract infections and herpes zoster (HZ), and occurred mainly in older patients with grade 3 lymphopenia. In most cases, it was necessary to discontinue the treatment with DMF due to the infection. It has been recently reported the first two young, not immunodeficient patients with normal ALC, who manifested severe HZ while on DMF (Anagnostouli et al., 2021). Authors suggest that the low CD8+ cells triggered HZ reactivation while the presence of increased NK cells facilitated an extensive manifestation, highlighting the potential role of T-cell subset monitoring. In our series, a young woman developed HZ in T5 dermatome while ALC $\geq$ LLN but she had been previously lymphopenic. The rest of patients with HZ had significant lymphopenia at the time of the infection.

In the present study, 22% of lymphopenic patients discontinued DMF due to persistent grade 2,3 lymphopenia. The time until ALC recovery was highly variable among patients, ranging from 1 month to 24 months. A recent observational study found that lower ALC count at DMF interruption, longer disease duration and previous exposure to other DMTs, delay ALC reconstitution (Lucchini et al., 2021).

The current study was subject to inherent limitations associated with integrating data from the clinical practice at different hospitals with variations in follow-up protocols, ALC monitorization and data acquisition procedures. In addition, low number of patients with grade 3 lymphopenia makes it difficult to identify significant differences in effect of previous immunosuppressive treatment. Lastly, although concomitant therapies that may affect lymphocyte counts (e.g., carbamazepine, steroids) were spontaneously reported by clinicians whenever deemed appropriate, this information was not routinely recorded and could therefore be considered a limitation of the study. Despite these limitations, this real-world population study with an increased sample size with respect to previous studies provides an easy tool for the early identification of patients at risk of developing lymphopenia under DMF therapy.

5. Conclusion

Patients who experience a reduction in ALC $>$ 21% within the three first months of DMF treatment have a 6.5-fold risk of development significant lymphopenia. If this reduction in ALC reaches 40% or more, the

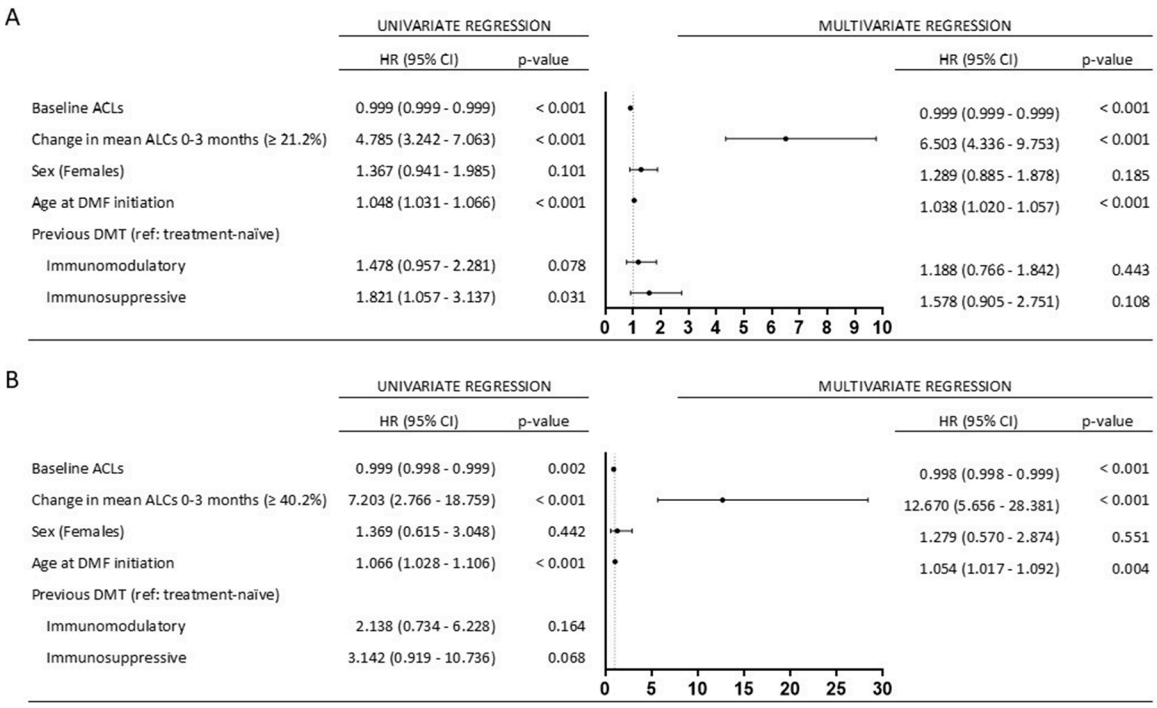

Fig. 4. Cox regression model: risk of significant (4a) and severe (4b) lymphopenia. Abbreviations: ALC, absolute lymphocyte count; DMF, dimethyl fumarate; DMT, disease modifying therapy.

Table 2
Lymphopenia related infections.

Patient	Sex	Age at DMF initiation (years)	Type of infection	Time after DMF initiation (months)	Duration of lymphopenia until infection symptoms onset (months)	ALC at the time of infection (cells/μl)	Infection management			Outcome
							Required hospitalization	Antimicrobial treatment	DMF discontinuation	
Patient 1	Female	65	Klebsiella pneumoniae UTI and aspiration pneumonia	15	12	300	Yes	Meropenem plus amikacin	Yes	Complete recovery
Patient 2	Female	22	Herpes zoster in right T5 dermatome	24	21	1100*	No	NA	No	NA
Patient 3	Male	41	Orofacial herpes zoster	12	6	400	No	Famciclovir	Yes	Postherpetic neuralgia
Patient 4	Female	44	Pneumococcal pneumonia	12	9	490	No	Levofloxacin	Yes	Complete recovery
Patient 5	Female	51	Herpes zoster in a thoracic dermatome	9	3	360	No	Valaciclovir	Yes	Postherpetic neuralgia
Patient 6	Female	49	Extensive herpes zoster over T1-L5 right dermatomes	10	1	800	No	None°	Yes	Complete recovery
Patient 7	Male	28	Bacterial pneumonia	1	0	1600	No	Amoxicillin/ clavulanic acid	No	Complete recovery

* ALC was normal at the time of herpes zoster but ALC within previous 6 months were below the lower limit of normality (nadir 800 cells/μl)
°No treatment was initiated because no medical advice was sought until skin lesions recovery.
ALC: absolute lymphocyte count; DMF: dimethyl fumarate; NA: not available; UTI: urinary tract infection.

risk of development severe lymphopenia is the multiplied by 12.7. We suggest closely monitoring older patients who have lower baseline ALCs and/or experience a rapid decrease in ALCs during the first 3 months of treatment. The remaining patients are unlikely to developed significant or severe lymphopenia if ALCs remain above the lower limit of normality during the first year. This data provides an easy tool for the monitoring and early identification of patients at risk of developing lymphopenia while on DMF.

Declarations

Funding

No funding was received for the design or conduct of the study, or the data analysis or interpretation of the results.

Ethical approval

This study was approved by The Ethical Committee of the Getafe University Hospital (Madrid, Spain), the Coordinator Center of the study.

Consent to participate

Informed consent was obtained from each participant.

Availability of data and material

Qualified researchers may request access to study protocol, statistical analysis and patient level data. Patient's data will be anonymized in order to protect the privacy of the participants.

CRediT authorship contribution statement

Susana Sainz de la Maza: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Julia Sabin Muñoz:** Conceptualization, Methodology, Validation, Investigation, Writing – review & editing. **Belén Pilo de la Fuente:** Investigation, Writing – review & editing. **Israel Thuissard:** Formal analysis, Writing – review & editing. **Cristina Andreu-Vázquez:** Formal analysis, Writing – review & editing. **Victoria Galán Sánchez-Seco:** Investigation, Writing – review & editing. **Paula Salgado-Cámara:** Investigation, Writing – review & editing. **Lucienne Costa-Frossard:** Investigation, Writing – review & editing. **Enric Monreal:** Investigation, Writing – review & editing. **Lucía Ayuso-Peralta:** Investigation, Writing – review & editing. **Lorena García-Vasco:** Investigation, Writing – review & editing. **José Manuel García-Domínguez:** Investigation, Writing – review & editing. **María Luisa Martínez-Ginés:** Investigation, Writing – review & editing. **Carmen Muñoz Fernández:** Investigation, Writing – review & editing. **Judit Díaz-Díaz:** Investigation, Writing – review & editing. **Celia Oreja-Guevara:** Investigation, Writing – review & editing. **Mayra Gómez-Moreno:** Investigation, Writing – review & editing. **Hugo Martín:** Investigation, Writing – review & editing. **Laura Rubio-Flores:** Investigation, Writing – review & editing. **María Rosario Blasco:** Investigation, Writing – review & editing. **Luisa María Villar-Guimerans:** Investigation, Writing – review & editing. **Yolanda Aladro:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – review & editing, Visualization, Supervision.

Declaration of Competing Interest

SSM has received honorary for speaking engagements or consulting services from Almirall, Biogen, Merck, Novartis, Roche, Sanofi-Genzyme and Teva. JSM has received funding for research projects and conference fees, mentoring, and assistance for conference attendance from Teva, Merck, Biogen, Roche, Novartis, and Sanofi. PBF, IT, CAV, VGSS, and PSC report no disclosures relevant to the manuscript. LCF received speaker fees, travel support, and/or served on advisory boards by Biogen, Sanofi, Merck, Bayer, Novartis, Roche, Teva, Celgene, Ipsen, Biopas, and Almirall. EM has received compensation for consulting services or participation in advisory boards from Sanofi, Biogen-Idec, Novartis and Roche; research support from Merck-Serono; travel expenses for scientific meetings from Almirall, Sanofi, Novartis, Roche, Merck-Serono and Biogen-Idec; and speaking honoraria from Sanofi, Biogen-Idec, Roche and Merck-Serono. LAP and LGV report no disclosures relevant to the manuscript. JMGD has received honoraria as speaker or consultant for Biogen, Sanofi, Abbvie, Bristol-Myers-Squibb, Merck, Roche, Almirall, Novartis and Teva pharmaceuticals. MLMG has received compensation for consulting services and speaking fees from Merck, Biogen, Novartis, Sanofi-Genzyme, Almirall, ROCHE, BMS, Bayer and Teva. CMF has received honoraria for speaking engagements from Novartis, Sanofi-Genzyme, Roche, Merck, and Biogen. JDD reports no disclosures relevant to the manuscript. COG has received speaker and consultation fees from Biogen Idec, Celgene, Sanofi-Genzyme, Novartis, Roche, Merck, and Teva. MGM, HM, and LR report no disclosures relevant to the manuscript. MRB reports no disclosures relevant to the manuscript. LMVG received research grants, travel support, or honoraria for speaking engagements from Biogen, Merck, Novartis, Roche, Sanofi-Genzyme, Celgene, and Bristol-Myer. YA has received funding for research projects or in the form of conference fees, mentoring, and assistance for conference attendance from Bayer, Biogen, Roche, Merck, Novartis, Almirall and Sanofi.

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