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Hematological Alterations Related to Treatment with Teriflunomide and Dimethyl Fumarate in Multiple Sclerosis

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Abstract: The exact mechanism of action of different modifying treatments in the evolutionary course of multiple sclerosis (MS) remains unknown, but it is assumed that they act upon the cells involved in acquired immunity. One effect of these treatments is the development of lymphopenia, which carries inherent safety risks. This study was conducted to understand the alterations that teriflunomide (TERI) and dimethyl fumarate (DMF) exert upon white blood cells in a series of patients with MS. This study included a total of 99 patients; 44 treated with DMF and 55 patients treated with TERI. Blood counts were evaluated at baseline and every 6 months in order to track the absolute leukocyte, lymphocyte, and neutrophil counts. Twelve months after starting treatment, we observed a significant decrease in leukocytes (21.1%), lymphocytes (39.1%), and neutrophils (10%) in the DMF group. In the TERI group, leukocytes decreased by 11.1%, lymphocytes by 8.1%, and neutrophils by 15.7%. Both TERI and DMF produced a significant decrease in leukocytes during the first year of treatment and this was mainly related with a decrease in neutrophils in the TERI group and a decrease in lymphocytes in the DMF group.

Keywords: teriflunomide; dimethyl fumarate; multiple sclerosis; lymphopenia; immunosuppressants

1. Introduction

Multiple Sclerosis (MS) is a demyelinating inflammatory disease of the central nervous system (CNS) with an autoimmune etiopathogenesis which involves both innate immunity (monocytes, neutrophils, macrophages, and dendritic cells) and acquired immunity directed towards a specific antigen (B and T lymphocytes and natural killer cells). The role of lymphocytes in the pathophysiology of MS is complex because the actions of both B and T lymphocytes are different but interrelated; B lymphocytes can activate T lymphocytes which, in turn, can activate B lymphocytes to cause the secretion of antibodies [1–3].

Every treatment used to modify the evolutionary course of MS affects adaptive immunity, that is, B and T lymphocytes, among others. Specifically, teriflunomide (TERI) inhibits the production of T and B cells, resulting in a 15% decrease in the white blood cell count (especially lymphocytes and neutrophils), which usually occurs in the third month of treatment but subsequently remains stable [4,5]. Dimethyl fumarate (DMF) reduces the percentage of T cells and of every B cell population type present in peripheral blood. Regarding the effects of DMF on T lymphocytes, it disproportionately decreases CD8+ lymphocytes compared to CD4+ lymphocytes, which translates into an increase in the CD4/CD8 ratio [4,5].

The objective of this current study was to verify the influence of treatment with DMF and TERI on different elements of the white blood cells, especially on lymphocytes, in a series of patients with MS,

and to compare our data with those published in the scientific literature from both clinical trials and studies performed in routine clinical practice.

2. Materials and Methods

2.1. Patients

We included patients with MS who had received treatment with TERI or DMF for at least 6 months, whose treatment with these drugs had either continued or had been withdrawn, regardless of the reason (the presentation of adverse effects or therapeutic failure). For patients who had switched between TERI or DMF therapy at some point, only the current treatment was considered in this study. All of the patients' white blood cell (WBC) counts were reviewed in order to obtain their baseline leukocyte parameters prior to the start of treatment, and thereafter WBC counts ($\times 10^9$/L) were performed every 6 months for leukocytes, lymphocytes, and neutrophils; when available, the CD4 and CD8 T lymphocyte subpopulations were also recorded.

2.2. Lymphopenia

The lowest value registered in the different analytics available was considered. An absolute count of 1000×10^9/L lymphocytes was considered as the lower limit of normality (LLN). The recommendations of the Common Terminology Criteria for Adverse Events were followed to classify the degree of lymphopenia as follows: grade-I: LLN-800×10^9/L lymphocytes; grade-II: 800–500×10^9/L lymphocytes; grade-III: 500–200×10^9/L lymphocytes; and grade-IV: $<200 \times 10^9$/L lymphocytes.

2.3. Statistics

Statistical analysis was performed with the SPSS statistical software package (IBM Corp., Armonk, NY, USA). The normality of the variables was verified using the Kolmogorov—Smirnov (goodness-of-fit) test. Normally distributed variables were assessed using parametric statistical tests (the mean, standard deviation, and Student *t*-test for independent or paired samples, as appropriate) and otherwise, non-parametric tests were used (the median, range, and Mann—Whitney U and Wilcoxon tests). Differences were considered to be statistically significant when the probability value was less than $p < 0.05$.

2.4. Ethical Issues

This study was approved by the ethics committee at our research institute in Galicia. In a scheduled consultation the patients gave their written informed consent to participate in this work and for the use of their records and subsequent clinical treatment data. Analytical data were recorded on a pseudo-anonymized form.

3. Results

A total of 99 patients were included in this study; 55 were treated with TERI and 44 received treatment with DMF. Sex, age, and previous treatments are shown in Table 1.

Table 1. Sex, age, and previous treatments in our series of MS patients.

	TERIFLUNOMIDE (n = 55)	**DIMETHYL FUMARATE (n = 44)**
SEX (M/F)	9 M/46 F	12 M/32 F
AGE (years)		
Mean ± SD *	49.4 ± 8.6	38.1 ± 8.5
Median (range) *	48 (32–73)	35.5 (25–55)
75th percentile *	56	45
PREVIOUS TREATMENTS		
Naïve	14	20
Glatiramer acetate	11	10
Interferon-β 1b	2	4
Interferon-β 1a sc	12	7
Interferon-β 1a im	7	1
Azathioprine	1	
Natalizumab	1	
Teriflunomide		2
Dimethyl fumarate	7	

* $p < 0.0001$.

In the DMF group, there was a decrease of 21.1% and 16.5% between the baseline values and the results at 12 months or 24 months, respectively, in the absolute numbers of leukocytes ($p < 0.001$) and a decrease of 39.1% and 30.8%, respectively, for the lymphocytes ($p < 0.001$). However, there was no significant effect on neutrophils. The decreases were more moderate in the TERI group with an 11.1% and 14.5% decrease in leukocytes 12 and 24 months after starting the treatment ($p < 0.01$), an 8.1% and 16.5% decline in lymphocytes ($p < 0.05$), and a 15.7% and 18.1% drop in neutrophils ($p < 0.01$). In the TERI group, 69.1% of the patients (n = 38) did not present lymphopenia, 16.4% (n = 9) presented grade-I lymphopenia, 12.7% (n = 7) had grade-II lymphopenia, and one patient showed grade-III lymphopenia (1.8%). All these lymphopenias were transitory and were not sustained over time.

In the DMF group, 50.0% of the patients did not have lymphopenia, 25% (n = 11) had grade-I lymphopenia, 18.2% (n = 8) showed grade-II lymphopenia, and three patients (6.8%) presented grade-III lymphopenia. In contrast to patients treated with TERI, lymphopenias after DMF were sustained throughout the treatment. DMF was withdrawn in 7 patients (15.9% of the total) because of the presentation of lymphopenia, either because it was grade III or because there was a >40% decrease in the value of the baseline counts (from 41% to 81%) in the case of grade-II lymphopenia.

Absolute white blood cell, lymphocyte, and neutrophil counts in patients treated with TERI or DMF are shown in Figure 1.

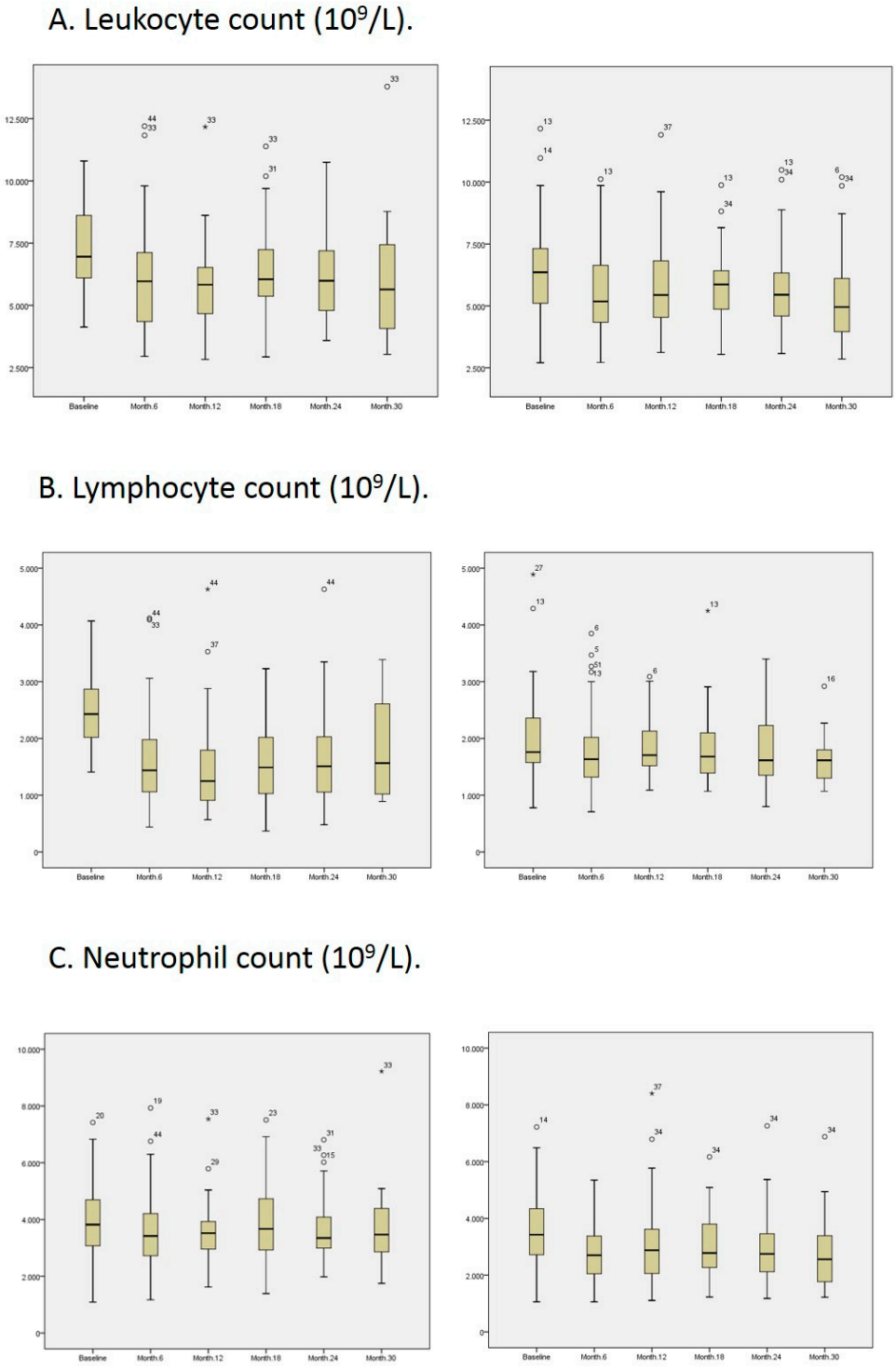

Figure 1. SPSS boxplot showing the effect of dimethyl fumarate (DMF) (left) and teriflunomide (TERI) (right) on white blood cell counts. Data is shown at baseline, 6 months, 12 months, 18 months, 24 months, and 30 months. In each period the number of patients treated with DMF was 44, 44, 43, 29, 23, and 18, respectively; and in the TERI group there were 55, 55, 54, 38, 34, and 26 patients, respectively. (**A**) Absolute leukocyte count. (**B**) Absolute lymphocyte count. (**C**) Absolute neutrophil count. The error bars represent the 95% confidence interval. The decrease in the mean leukocyte count during the first year of treatment is influenced by a decrease in the neutrophil count in the TERI group and by lymphopenia in the DMF group.

A sharp drop in leukocytes was observed 6 months after starting treatment with either drug, although this decrease was more marked for DMF; in both cases the effect lasted up to 12 months, and thereafter remained stable. The decrease in leukocytes observed in the TERI group can be explained by a reduction in neutrophils, while the reduction in the DMF group could be explained by a decrease in lymphocytes.

The CD4+ and CD8+ lymphocyte subpopulations were determined in a subgroup of 22 and 33 patients treated with DMF or TERI, respectively. In these groups we observed a trend towards a higher CD4/CD8 ratio for the patients receiving DMF treatment, although this did not reach statistical significance (3.25 ± 1.63 vs. 2.59 ± 1.01, $p = 0.056$).

4. Discussion

A pooled analysis of the results from clinical trials which assessed the use of TERI indicates that the absolute lymphocyte count fell by 22% in these patients in the first 24 weeks but remained stable for the rest of their treatment. Of note, grade-I lymphopenia occurred in 7.3% of these patients and 2.2% had grade-II lymphopenia [6]. To adequately compare the data we obtained in our patient group treated with TERI to the results previously reported by Comi et al. [6], it is important to consider at least two consecutive determinations with lymphopenia. Thus, the percentages in our series were modified as follows: 85.6% of patients being lymphopenia-free, grade-I or II lymphopenia being observed in 14.4% (7.2% grade-I and 7.2% grade-II), and finally, no cases of grade-III lymphopenia being detected. In general, our data from patients treated with TERI are consistent with previously reported results. Our study also showed that 6 months after starting the regimen there was a 33.3% decrease in neutrophils among patients treated with TERI, with this reduction stabilizing at around 20% in the following months without the withdrawal of the treatment in any case.

In the interim analysis at 5 years, the ENDORSE study (a 12-year observational study on the efficacy and safety of DMF, which included patients from the DEFINE [7] and CONFIRM [8] clinical trials as well as new patients) reported that the prevalence of patients with grade-III lymphopenia (200 to 500 lymphocytes) was 7.5% [9]. In real-life studies, and in agreement with our findings, the prevalence of lymphopenia in patients treated with DMF seemed to be somewhat higher, with grade-II lymphopenia representing 17–20% of treated patients. It is also important to recognize that the recovery from lymphopenia can be slow, which often translates into an impasse in terms of the therapeutic decisions taken a posteriori [10]. These authors found that a low baseline lymphocyte count was a predictor of developing grade-II or III lymphopenia during treatment with DMF.

Another study that evaluated the risk of lymphopenia associated with DMF treatment found that 17% of patients had grade-II–III lymphopenias, and that this complication was related to older patient age (9.5% in patients aged <40 years, 19% when aged 40–55 years, and 27% in patients aged over 55 years) as well as previous exposure to natalizumab. In addition, they also found that lymphopenia did not normalize during the course of treatment with DMF [11]. Other studies have also found that the time required for recovery from lymphopenia associated with DMF treatment was related to both the age of the patients [12] and the duration of lymphopenia before discontinuation of the treatment [13].

Monitoring the lymphocyte count is key to predicting the development of severe lymphopenia. In this sense, it has been suggested that a decrease in lymphocytes by more than 38% by the third month of treatment with DMF put patients at a 6-fold higher risk of developing grade-II–III lymphopenia [14], while patients who maintained a lymphocyte count above the LLN (>1000 lymphocytes) for the first 6 months (84%) or the first 12 months (76%) developed grade-III lymphopenia (<500 lymphocytes) in only 0.1% and 0% of cases, respectively [15].

Very few cases of progressive multifocal leukoencephalopathy (PML) have been reported in patients treated with fumaric acid esters, either among those with psoriasis or MS. Moreover, to date, just seven cases of PML related to treatment with DMF have been reported in the academic literature, representing a prevalence of 0.02 cases/1,000 patients treated [16]. The possibility of developing PML would also be reflected in routine clinical practice whereby patients undergoing DMF treatment are a

decade younger than those completing regimens with TERI, a practice—or perhaps a bias—associated with the age and immunosenescence binomial.

Are these hematological abnormalities important in clinical practice for choosing treatment? In addition to the high efficacy shown in clinical trials of DMF in reducing both the rate of relapses and the activity in magnetic resonance imaging [7,8], several real-life studies based on propensity-score matching techniques to eliminate selection biases and homogenize groups, seem to show that DMF is a better treatment than TERI both in terms of clinical and radiological disease activity [17–19]. Thus, we could conclude that DMF should be the first option considered when deciding to start treatment with a first-line drug. However, there is also evidence for an increased age-associated risk of immunosuppression when using DMF and a possible problem arises involving the safety of the treatment. From the natural history of MS it is well known that relapses decrease throughout the course of the disease, thus translating into lower inflammation during more advanced disease stages, and then the patients would not need to be treated with a highly potent disease-modifying treatment. In this sense, TERI would not affect the absolute lymphocyte count of patients, being therefore safer. On a more practical level, treatment switching for the convenience of patients who have remained clinically and radiologically stable for years should favor the indication for TERI because this drug has a similar efficacy to that of injectable treatments but a better safety profile than DMF when considering the prevalence of lymphopenias [20,21].

In summary, our study shows that patients treated with TERI do not present clinically relevant lymphopenias while patients undergoing treatment with DMF present lymphopenias which require the discontinuation of this drug in 15% of cases. Both treatments cause a reduction in the absolute leukocyte count, but there is a qualitative difference between the two; with TERI the reduction is mainly found in the neutrophil count while DMF tends to result in a lymphopenia.

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