MSJ Experimental Translational Clinical

Original Research Paper

A retrospective analysis of changes in lymphocyte levels in patients with multiple sclerosis during and after Tecfidera® treatment

Multiple Sclerosis Journal—Experimental, Translational and Clinical

July–September 2021, 1–10

DOI: 10.1177/20552173211029674

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Abstract

Background: There are currently no best practice recommendations for lymphocyte subset monitoring for patients with multiple sclerosis (pwMS) on disease-modifying therapies including Tecfidera® (dimethyl fumarate, DMF). However, there have been several cases of pwMS on DMF without severe lymphopenia who had high CD4:CD8 T cell ratios and went on to develop progressive multifocal leukoencephalopathy.

Objective: Our objective was to characterize the changes in immune profile during and after DMF treatment in pwMS.

Methods: A retrospective analysis of longitudinal data from 299 pwMS who have been treated with DMF at the Fraser Health Multiple Sclerosis Clinic in British Columbia, Canada. The blood test results were taken from January 1, 2013 to April 1, 2020.

Results: Our results suggest that CD8+ T cells had the highest proportional decrease compared to other lymphocyte subset populations and overall lymphocyte count in response to DMF treatment. CD56+ Natural Killer cells were similarly decreased in response to DMF treatment. CD4:CD8 T cell ratio was the measurement that had the highest rate of change in response to DMF initiation and discontinuation.

Conclusion: CD8+ T cell count and CD4:CD8 T cell ratio may be a more sensitive measurement of the immune landscape of patients with MS on DMF.

Keywords: Multiple sclerosis, allergy and immunology, lymphocytes, dimethyl fumarate, t-lymphocytes, killer cells, natural

Date received: 3 March 2021; accepted: 14 June 2021

Introduction

There are currently no best practice recommendations for lymphocyte subset monitoring for patients with multiple sclerosis (pwMS) on disease-modifying therapies (DMTs), including Tecfidera® (dimethyl fumarate, DMF). Many previous studies have demonstrated a total decrease in absolute lymphocyte count (ALC) in patients on DMF[1–8] and this measurement is used to track their immune status. Studies exploring changes in specific subpopulations of lymphocytes in patients treated with DMF found significant decreases in CD8+ T cells.[3–5,8–12] Since a positive correlation was found between changes in ALC and specific lymphocyte populations,[5] sub monitoring of lymphocytes has not been recommended.[3,13] However, there have been several cases of pwMS on DMF without severe lymphopenia who had high CD4:CD8 T cell ratios and went on to develop progressive multifocal leukoencephalopathy (PML),[14–16] a potentially fatal infection caused by John Cunningham virus (JCV) reactivation. These cases suggest that monitoring changes in lymphocyte subpopulations remains clinically relevant and important.

We performed a retrospective analysis on lymphocyte changes in pwMS on DMF hypothesizing that

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we would find a decrease in CD8+ T cell and NK cell populations which would be reflected more accurately by CD4:CD8 T cell ratio rather than ALC. This information can potentially lead to clinical practice improvement by detecting lymphopenia and relative risk of infection that would not be captured by traditional ALC.

Methods

We performed a retrospective database search from several electronic medical record systems of pwMS who have been treated with DMF between January 1, 2013 to April 1, 2020 at the Fraser Health Multiple Sclerosis Clinic in Burnaby, British Columbia, Canada. Our inclusion criteria included patients who were over the age of 18 and had a confirmed diagnosis of relapsing-remitting multiple sclerosis (RRMS) as determined by the 2017 McDonald Criteria for the Diagnosis of MS. In total, 299 patients have been identified as being treated with DMF and were used for demographic analysis. Blood tests were performed at either Vancouver General Hospital or St. Paul's Hospital which both provide peripheral lymphocyte counts for our region. Eleven patients did not have blood tests accessible to our clinic after beginning DMF treatment and were removed from further analyses. Blood tests were drawn approximately 6 months prior to treatment start and were regularly scheduled every three months. For immune cell changes during DMF treatment, the baseline ($x = 0$) chosen was the nearest blood test prior to treatment start date. Our cohort included patients on DMF up until 77 months post treatment start date (cut off date for analysis: April 1st, 2020). However, given the reduced number of patients that remain on DMF for longer than 40 months, there was increased variability at the later time points across all blood cell counts and therefore 31 months was selected as the end point, being the average treatment duration time. For immune cell changes after DMF discontinuation, the baseline ($x = 0$) chosen was the last blood test prior to treatment end date.

Individual cell counts were plotted with respect to time since treatment initiation or discontinuation. Next, the average cell count mean and standard deviation was calculated at each month to give an average change in immune cell subset populations over time. Normal blood cell count/ratio was based on guidelines from the hospitals analysing the blood tests. Statistical analysis was performed using GraphPad Prism 7.05 software. Linear regression and one-way ANOVA were performed. For change in cell count during treatment, mean magnitude and percent change of cells was calculated based on average starting levels at baseline (prior to treatment start) and at 31 months post treatment start date. For change in cell count after treatment discontinuation, mean magnitude and percent change in cell count was calculated based on linear regression equation derived from best fit linear slope. Predicted average time until cell counts are out of normal range (low or high) and time until recovery to prior treatment levels were also calculated based on linear regression equation.

A co-chair or delegated member of the Fraser Health Research Ethics Board (FHREB) has reviewed and approved this study in accordance with the FHREB Policy on Ethical Conduct of Research and Other Studies Involving Human Participants, the Tri-council Policy Statement: Ethical Conduct for Research Involving Humans, and the International Conference on Harmonisation Guidance E6: Good Clinical Practice E6: Consolidated Guidelines.

This data has been approved for publication that comply with internal Fraser Health Authority policy and the International Committee of Medical Journal Editors (ICMJE) guidelines. Study protocol and statistical analysis plan are available. The data is available upon publication and ending 5 years following article publication. Only researchers who provide a methodologically sound proposal will have access to the de-identified study data. Proposals should be directed to REB@fraserhealth.ca. To gain access, data requestor will need to sign a data access agreement.

Results

Demographics

A total of 299 pwMS treated with DMF were used for the study and their demographic data is presented in Table 1. In summary, our cohort was 69% female and a median 42 years old. The majority of our cohort had no prior DMT use (57%), while those with prior DMT treatment were most likely to have used glatiramer acetate (Copaxone®) (12%). Median time spent on DMF as of April 1st, 2020 was calculated to be 26 months or 2.2 years. Age and treatment duration were not normally distributed although mean and median were relatively similar for both age (42.1 and 42.0 years, respectively) and treatment duration (2.2 and 2.6 years, respectively) (Supplementary Figure S1). This demographic is

Table 1. Demographic characteristics of pwMS prescribed DMF.

Demographic characteristics	Number (%)
Total patients	299
Ongoing DMF treatment[a]	151 (51%)
Stopped DMF treatment	148 (49%)
Female patients	205 (69%)
Caucasian[a]	286 (96%)
Non-Caucasian[b]	14 (4%)
Median start age of patients for DMF treatment	42 ± 10.5 years
Median time on DMF treatment[a]	2.6 ± 1.8 years
Patients removed from analysis (no blood tests)	12 (4%)
Patients with complete blood count (CBC) differential data	46 (15%)
Patients with CBC + T cell subtype data	31 (10%)
Patients with CBC + full lymphocyte subset data	211 (71%)
No prior DMTs	171 (57%)
One prior DMTs	93 (31%)
Aubagio	1 (0.3%)
Avonex/AvoPen	16 (5%)
Betaseron	10 (3%)
Mavenclad	1 (0.3%)
Copaxone	36 (12%)
Gilenya	1 (0.3%)
Minocycline	1 (0.3%)
Rebif	23 (8%)
Tysabri	4 (1%)
Two prior DMTs	29 (10%)
Three or more prior DMTs	6 (2%)

[a]Caucasian includes those who self-identify as white, European, Indian, Persian and Mediterranean.
[b]Non-Caucasian includes those who self-identify as Asian, mixed race, Latinx, and Caribbean.

similar to previous studies looking at lymphocyte subset population changes in pwMS on DMF.

Changes in immune cell subpopulations during treatment

Our results show that in pwMS after 31 months on DMF treatment, overall white blood cell count is decreased (-1.99×10^9 cells/L, -29.1%). However, this is a proportionally smaller decrease than ALC or its subsets and would predictably only cross the lower threshold after 44.9 months of treatment (Table 2, Figure 1(a)). ALC has a 49.2% decrease (-1.02×10^9 cells/L) which would fall out of normal range after 26 months of treatment (Table 2, Figure 1(c)). Proportionally similar changes were seen in CD4+ T cell counts (-0.51×10^9 cells/L, -48.5%) (Table 2, Figure 1(d)) but would fall out of normal range later at 30.1 months post treatment initiation. Our analysis shows that out of all the lymphocytes, CD8+ T cells are the first to decrease past normal range (Table 2, Figure 1(e)) with a mean decrease of -0.34×10^9 cells/L (-67.0%) after 21.1 months of treatment. NK cells also had a significant decrease (-0.08×10^9 cells/L, -52.5%) but by our calculations would not reach the lower threshold until 34.2 months (Table 2, Figure 1(g)). B cell counts were also decreased (-0.08×10^9 cells/L, -29.3%) (Table 2, Figure 1(f)). There were no significant changes in neutrophil counts (Table 2, Figure 1(b)). The CD4:CD8 T cell ratio had a high proportional increase (1.01, 41.8%) and was the marker most quickly to exit out of normal range at 10.6 months post treatment start date (Table 2, Figure 1(h)). Sex did not appear to play a significant role in most immune cell counts during DMF treatment (Supplementary Table S1, Figure S2). Changes in CD4+ T cell count and CD4:CD8 T cell ratio appeared to be significantly different between the different age cohorts. As patients got older, CD4+ T cells decreased and CD4:CD8 T cell ratio increased at a greater rate. Other immune cells were not significantly affected (Supplementary

Table 2. Changes in immune cell counts/ratio during DMF treatment.

Cell types	Normal range ($\times 10^9$ cells/L or ratio)	Magnitude change ($\times 10^9$ cells/L or ratio) per month	Mean magnitude change ($\times 10^9$ cells/L or ratio) after 31 months, n (%)	Time until out of normal range (months)	ANOVA p-value	Linear trend p-value
White blood cell	4.0–11.0	–0.06384/month	–1.99 (–29.1%)	44.9	**<0.0001**	**<0.0001**
Neutrophils	2.0–8.0	–0.0187/month	–0.82 (–20.2%)	109.4	0.1579	**0.0102**
Lymphocytes	1.0–4.0	–0.04125/month	–1.02 (–49.2%)	26.0	**<0.0001**	**<0.0001**
CD4+ T cells	0.41–1.33	–0.02131/month	–0.51 (–48.5%)	30.1	**<0.0001**	**<0.0001**
CD8+ T cells	0.20–0.78	–0.01463/month	–0.34 (–67.0%)	21.1	**<0.0001**	**<0.0001**
CD19+ B cells	0.07–0.50	–0.004174/month	–0.08 (–29.3%)	52.4	**0.0003**	0.3252
CD56+ NK cells	0.08–0.75	–0.002339/month	–0.08 (–52.5%)	34.2	**0.0007**	**0.0469**
CD4:CD8 T cell ratio	0.50–3.21	0.07363/month	1.01 (41.8%)	10.6	**<0.0001**	**<0.0001**

Table S2, Figure S3). Prior DMT treatment resulted in a 2-fold greater decrease in lymphocyte and CD4+ T cell count. There were no significant differences in other immune cell counts (Supplementary Table S3, Figure S4). Overall, our results confirm our hypothesis that CD8+ T cells are the first lymphocyte subpopulation to decrease outside the normal range and with the largest proportional change. This change is reflected in the overall CD4:CD8 T cell ratio, which may be a more sensitive measurement to reflect immune imbalance than ALC.

Changes in immune cell subpopulations after treatment discontinuation

To investigate immune cell recovery after treatment discontinuation, we analyzed two cohorts of patients for which blood tests post treatment were available: all patients that have discontinued Tecfidera (Table 3) and the subset with cell counts outside the normal range (Table 5). We performed a similar linear regression analysis to the changes in cell counts during treatment to determine time until recovery and magnitude and percent change up to 6 months post treatment discontinuation. When accounting for all patients, regardless of cell count at treatment discontinuation, we were only able to find a statistically significant trend in CD8+ T cell recovery (Table 3). For this immune cell subpopulation, there was a predicted increase of 0.18×10^9 cells/L (93.1%) after 6 months of treatment (Table 3) which was the highest proportional change out of all the immune cell subpopulations. Additionally, although not statistically significant, there appeared to be a continuing decrease in white blood cell and lymphocyte count during the chosen timeframe. We did not find significant differences in immune cell recovery between male and female patients, different age cohorts or prior DMT use after DMF treatment discontinuation (Supplementary Table S4-6, Figure S5-7).

The lack of statistically significant results in this population suggests that segregation between abnormal and normal cohorts at the end of treatment is necessary to present a more accurate immune landscape after treatment discontinuation, especially in patients who discontinued as a result of abnormal cell counts. We analyzed the subset of patients only with cell counts outside of normal ranges. Although the average normal range for ALC is $1.0–4.0 \times 10^9$cells/L, many physicians use 0.5 $x10^9$ cells/L (grade 3 lymphopenia) as the cut-off point for discontinuing DMF treatment, therefore two sub cohorts were created for our post treatment abnormal ALC analysis. Our results demonstrated that while only 33.3% of patients had an ALC below 1.0 $x10^9$ cells/L and out of those 8.5% had an ALC below 0.5 $x10^9$ cells/L, CD8+ T cell count had the highest number of patients with cell counts outside of normal range at treatment end date (55.0%), followed by CD4:CD8 T cell ratio (53.7%) and closely followed by NK cells (51.4%) (Table 4). Although NK cell recovery demonstrated a statistically significant linear trend, it did not reach significance per one-way ANOVA analysis (Table 5). On average, ALC recovery occurred after approximately 4 months, regardless of whether patients had a count below 0.5×10^9 cells/L or not (Figure 2(e) to (h)). Interestingly, B cells had the quickest recovery at 0.5 months (Figure 2(m) and (n)) while CD4+ and CD8+ T cells had similar recovery rates (3.5 and 3.7 months, respectively) (Figure 2(i) to (l)). Regarding percent change after 6 months of treatment discontinuation, although this result was not statistically significant, CD4:CD8 T cell ratio had

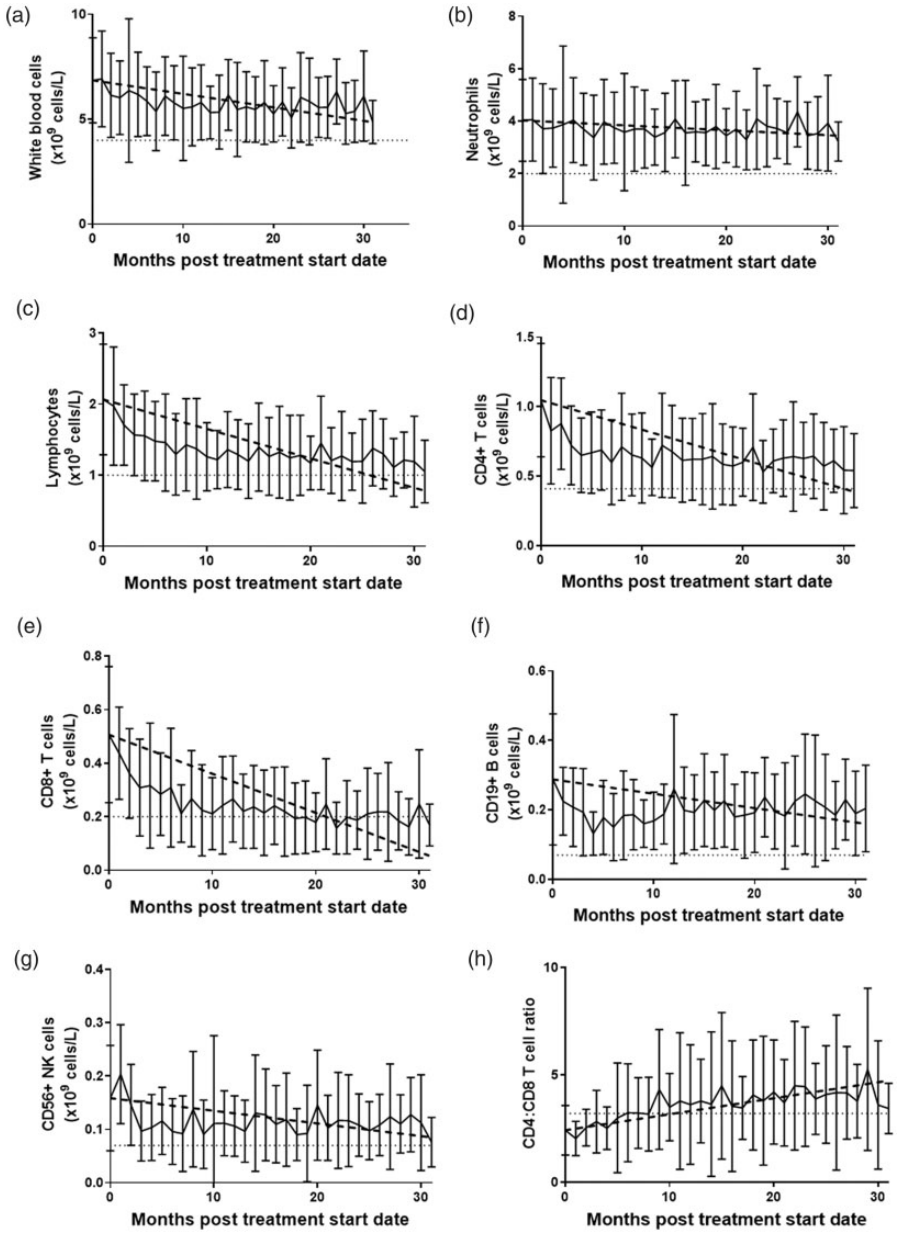

Figure 1. Graphs of average immune cell changes during DMF treatment. Mean white blood cell count (a), neutrophil count (b), ALC (c), CD4+ T cell count (d), CD8+ T cell count (e), CD19+ B cell count (f), CD56+ NK cell count (g), and CD4:CD8 T cell ratio (h) are plotted against time after treatment initiation. Dashed line represents linear regression slope. Dotted line represents normal range. Error bars represent standard deviation at each timepoint.

the lowest % change (−34.6%) and slowest recovery rate (7.3 months) compared to other immune cell counts (Table 5, Figure 2(q) and (r)). White blood cell count recovered after 3 months and neutrophils had a non-statistically significant recovery after 2 months post treatment termination (Figure 2(a) to (d)). Cumulatively, these results suggest that while lymphocyte, CD4+ and CD8+ T cell counts appear to recover at relatively the same rate, a higher percentage of patients had abnormal CD8+ T cells counts, NK cell count and CD4:CD8 ratio than abnormal lymphocyte count. There was no statistically significant difference found between male and female immune cell recovery in those with abnormal immune cell count after DMF discontinuation (Supplementary Table S7, Figure S8). Additionally, the discrepancy in recovery times between ALC, CD4+ T cells, CD8+ T cells and CD4:CD8 T cells suggests that the immune landscape takes longer to "re-balance" after DMF discontinuation

Table 3. Changes in immune cell counts/ratio of all patients after DMF discontinuation.

Cell types	Normal range ($\times 10^9$ cells/L or ratio)	Magnitude change ($\times 10^9$ cells/L or ratio) per month	Mean magnitude change after 6 months ($\times 10^9$ cells/L or ratio)	Percent change after 6 months	ANOVA p-value	Linear trend p-value
White blood cells	4.0–11.0	–0.03034/month	–0.18	–3.1%	0.7660	0.7890
Neutrophils	2.0–8.0	0.0006916/month	0.004	0.1%	0.7986	0.6414
Lymphocytes	1.0–4.0	–0.03980/month	–0.24	–25.1%	0.5009	0.0508
CD4+ T cells	0.41–1.33	0.02454/month	0.15	27.2%	0.3551	0.1284
CD8+ T cells	0.20–0.78	0.03023/month	0.18	93.1%	**0.0408**	**0.0111**
CD19+ B cells	0.07–0.50	0.01580/month	0.09	54.8%	0.2829	0.3865
CD56+ NK cells	0.08–0.75	0.008156/month	0.09	54.8%	0.2186	0.1716
CD4:CD8 T cell ratio	0.50–3.21	–0.2423/month	–1.45	–36.6%	0.3462	0.0977

Table 4. Abnormal lymphocyte subpopulation counts/ratio after dimethyl fumarate discontinuation.

Cell types	Total number of patients	Number of patients with abnormal cell counts	Percent of patients with abnormal cell counts
White blood cells	117	19	16.2%
Neutrophils	117	7	6%
Lymphocytes ($<1.0\times 10^9$ cells/L)	117	39	33.3%
Lymphocytes ($<0.6\times 10^9$ cells/L)	117	10	8.5%
CD4+ T cells	42	18	42.9%
CD8+ T cells	40	22	55.0%
CD19+ B cells	37	4	10.8%
CD56+ NK cells	35	31	51.4%
CD4:CD8 T cell ratio	41	39	95.1%

Table 5. Changes in immune cell counts/ratio of patients with abnormal cell counts after DMF discontinuation.

Cell type	Normal range ($\times 10^9$ cells/L or ratio)	Magnitude change ($\times 10^9$ cells/L or ratio) per month	Magnitude change after 6 months ($\times 10^9$ cells/L or ratio), n (%)	Time until back in normal range (months)	ANOVA p-value	Linear trend p-value
White blood cells	4.0–11.0	0.2505/month	1.50 (45.3%)	3	**0.0062**	**0.0297**
Neutrophils	2.0–8.0	0.2347/month	1.41 (92.2%)	2	0.2412	0.1389
Lymphocytes ($<1.0\times 10^9$ cells)	1.0–4.0	0.09652/month	0.58 (97.8%)	4.2	**<0.0001**	**<0.0001**
Lymphocytes ($<0.5\times 10^9$ cells)	1.0–4.0	0.1455/month	0.87 (235.9%)	4.3	**<0.0001**	**<0.0001**
CD4+ T cells	0.41–1.33	0.04456/month	0.27 (104.6%)	3.5	**0.0019**	**0.0003**
CD8+ T cells	0.20–0.78	0.03127/month	0.19 (224.7%)	3.7	**0.0071**	**0.0077**
CD19+ B cells	0.07–0.50	0.02211/month	0.13 (221.1%)	0.5	0.1988	**0.0247**
CD56+ NK cells	0.08–0.75	0.01145/month	0.07 (143.8%)	2.8	**0.0543**	**0.0037**
CD4:CD8 T cell ratio	0.50–3.21	–0.3192/month	–1.92 (–34.6%)	7.3	0.1841	0.0576

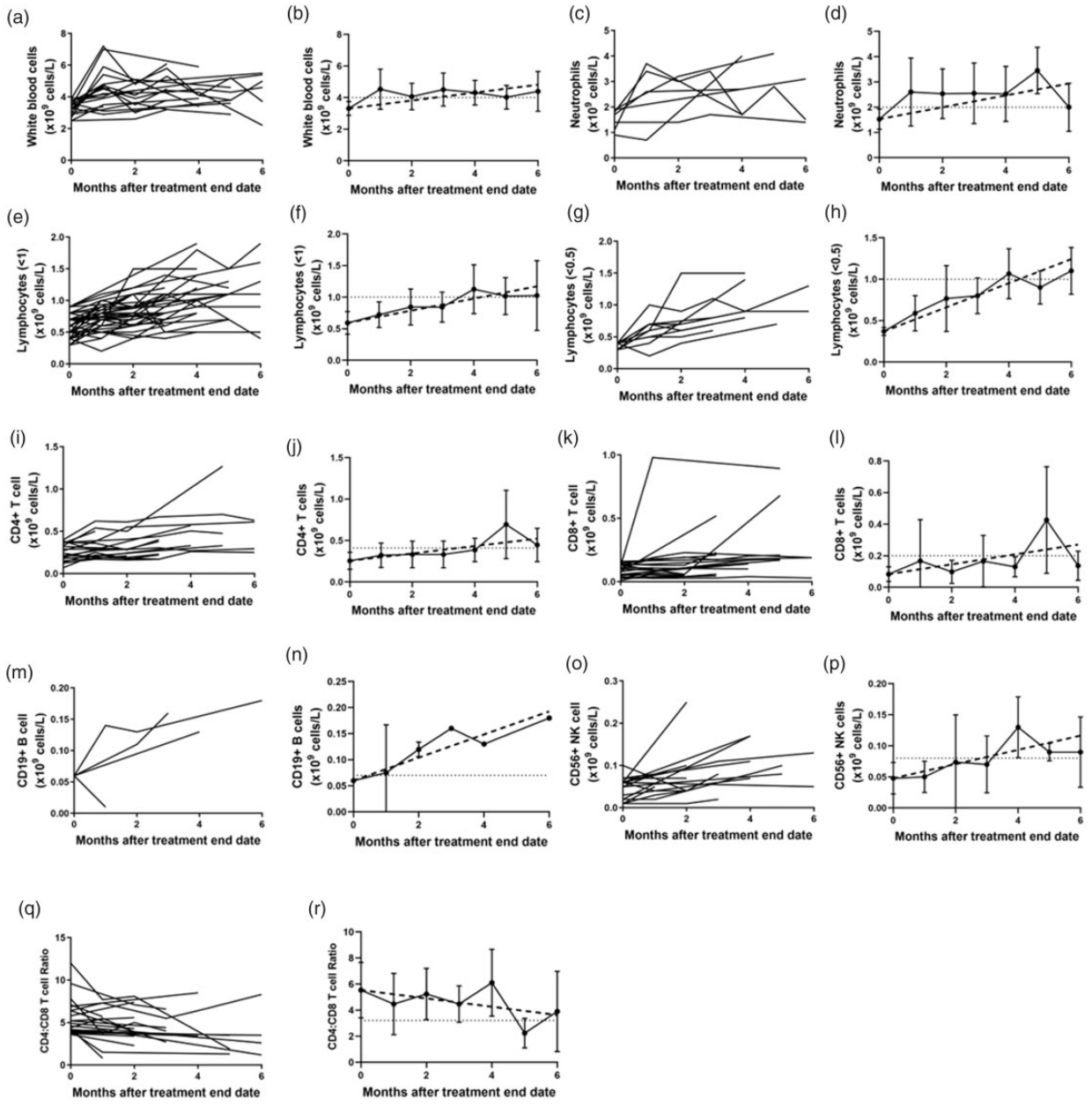

Figure 2. Graphs of changes in immune cell counts/ratio in patients with abnormal cell levels after DMF discontinuation. Mean for individual and average white blood cell count (a, b), neutrophil count (c, d), ALC below $1.0x10^9$cells/L (e, f), ALC below $0.5x10^9$cells/L (g, h), CD4+ T cell count (i, j), CD8+ T cell count (k, l), CD19+ B cell count (m, n), CD56+ NK cell count (o, p), and CD4:CD8 T cell ratio (q, r) are plotted against time after treatment discontinuation. Dashed line represents linear regression slope. Dotted line represents normal range. Error bars represent standard deviation at each timepoint.

than what would be expected if looking at individual immune cell subpopulations measurements.

Discussion

Our study was a longitudinal retrospective analysis investigating changes in immune cell populations in pwMS using DMF and quantifying that rate and magnitude of change over time. Previous studies have consistently shown decreases in ALC[2–8,12] as well as various subpopulations, notably demonstrating a higher decrease in CD8+ T cells compared to CD4+ T cells.[3–5,8,10,12,17] However, using ALC as a representative of change in all lymphocyte subsets has remained common practice.[3,13] The general recommendation is to discontinue the use of DMF after 6 months of chronically low ALC ($<0.5 \times 10^9$cells/L). There is no official recommendation for DMF

washout period, although it is common for physicians to wait until ALC is within normal range ($>1.0 \times 10^9$cells/L) before starting a new treatment. Many of the studies that suggest that subset monitoring is not required do not investigate whether the ALC was preferable to lymphocyte subset counts in terms of predicting lymphopenia risk or recovery. Additionally, it is still unclear whether lymphopenia is a better predictive marker of adverse events than CD8+ T cells, in particular with regards to infections.

After treatment start, we saw significant mean decreases in ALC and its subpopulations, including CD4+ T cells, CD8+ T cells, B cells and NK cells. We found that CD8+ T cells had the highest proportional change (−67.0%) and quickest decrease out of normal range (21.1 months). Our results appear to demonstrate that the changes in ALC are more accurately reflecting the changes in CD4+ T cells rather than CD8+ T cells and ALC change occurred over a longer period of time. When examining the cell counts of patients who had stopped DMF treatment, we found that 33.3% of patients had an ALC below 1×10^9cells/L while 8.5% had a count of below 0.5×10^9/cells/L. More than half of patients who discontinued DMF treatment had a CD8+ T cell count, NK cell count and/or CD4:CD8 T cell ratio outside of the normal range. When examining the blood test results of patients who discontinued DMF and who had abnormal cell counts, CD4+ T cell and CD8+ T cell recovery appears to occur at roughly the same time (3.5 and 3.7 months respectively) and slightly earlier than patients with ALC below 1×10^9 cells/L (4.2 months). Although not statistically significant, CD4:CD8 T cell ratio had the smallest change (−36.6%) 6 months post treatment discontinuation and a two-fold longer recovery time compared to individual immune cell subpopulations. Based on our analysis, sex did not appear to play a significant role in immune cell count during DMF treatment and recovery after discontinuation. Increased age was associated with decreased CD4+ T cell count and increased CD4:CD8 T cell ratio during DMF treatment. However, we could not determine that age affects immune cell recovery. Prior DMT treatment was associated with decreased lymphocyte and CD4+ T cell count during DMF treatment. We could not determine that prior DMT treatment affects immune cell recovery.

To date, the studies with the largest patient cohort and longest time course evaluating changes in immune profile in patients on DMF come from large clinical trials such as the phase III DEFINE[18] and CONFIRM[6] studies and the subsequent PROCLAIM[5] and ENDORSE[5,19,20] extensions. In the ENDORSE trial, ALC was primarily monitored and it was found that 60% of patients maintained an ALC above 0.91×10^9 cells/L over the 10-year study, with an average decrease and subsequent plateau of −33.6% by week 24 (5). In the PROCLAIM study, they found that B cells actually had the greatest rate and proportional decline, followed by reductions in CD3+CD4+ T cells, CD3+CD8+ T cells, CD3+ T cells and NK cells.[5] Similar to subsequent studies, including ours, they found that CD8+ T cell count was significantly more reduced compared to CD4+ T cells. When the same trial was analyzed with a specific focus on ALC, they found that 47/2,099 (2.2%) patients treated for over 6 months had an ALC less than 0.5×10^9 cells/L that lasted for 6 months.[7] Overall, they found that the majority of patients (76%, 1,876/2,470) had an ALC greater than 0.5×10^9 cells/L after 1 year of DMF treatment.[7]

There have been fewer studies examining immune cell recovery after DMF discontinuation. One study investigated ALC repopulation after treatment discontinuation in 11 RRMS patients who developed lymphopenia out of 246 DMF-treated patients.[21] Important to note that in this study, fewer patients (4.47%) developed lymphopenia compared to our study (8.5%). Despite this difference in proportion of patients who develop lymphopenia, they found that ALC is able to recover above 0.8×10^9cells/L after approximately 5 months, similar to our results.[21] Another study examined recovery of ALC, CD4+ T cell count and CD8+ T cell count 30-months post-DMF discontinuation, and separated the cohorts into two groups: those who did not subsequently take any DMTs or switched to a DMT with no known effect on lymphocyte count or those who switched to a DMT with known effects on lymphocyte counts.[22] They found that regardless of the DMT used after DMF discontinuation, there was no statistically significant recovery trend in any of the lymphocytes.[22]

Decreased immune cell counts leads to the question of increased infection rates. Due to the difficulty with patients' reporting infections retrospectively, we were unable to further analyze the link between infection rates and immune cell counts in this population. One of the key areas of concern regarding infections and DMF-associated decrease in

lymphocytes is the possibility of JCV reactivation and subsequent development of PML.[12] Given that PML is still a relatively rare event only recently described in patients taking DMF, our study did not analyze the link between low immune cell subpopulation count and PML rates. Although some cases studies have noted a link between chronic lymphopenia due to DMF and PML,[23,24] one study found that declining ALC did not correlate with anti-JCV antibody titers regardless of DMT.[25] Additionally, there have been cases of patients with no lymphopenia but increased CD4:CD8 T cell ratio developing PML.[14–16] In a meta-analysis examining the ALC, CD4+ T cell and CD8+ T cell counts in 19 patients with either psoriasis or MS who went on to develop PML and were treated with drugs where DMF is the main or sole ingredient, grade 1 or 2 lymphopenia was noted in 5 out of the 14 cases prior to PML onset.[26] In the 10 cases where CD4 and CD8+ T cell counts were available, the average counts were both below normal range at 0.155×10^9 cells/L and 0.052×10^9 cells/L, respectively.[26] However, it is important to note that the CD4+ and CD8+ T cell counts were done at the time of PML diagnosis and not prior and therefore it is unclear from this study whether decrease occurred before or after disease onset. When comparing PML survivors and PML progressors, one study found that JCV-specific CD8+ T-cell responses and not CD4+ T-cell responses were different between the two groups.[27] Given these studies, it may lend support to our recommendations for lymphocyte subset monitoring of T cells in patients on DMF for potential PML risk.

Conclusion

Our study provides real word evidence of a lag in ALC change compared to lymphocyte subpopulations in patients with MS treated with DMF. The cell count with the greatest rate and proportional change were CD8+ T cells while CD4:CD8 T cell ratio was the measurement most sensitive to DMF treatment. We suggest that measuring CD8+ T cell counts and CD4:CD8 T cell ratio in patients on DMF may better reflect the changing immune landscape and future studies may focus on analyzing whether these markers could serve as a predictive factor for DMT-associated infections such as PML. We hope this in-depth assessment leads to practice improvement by providing physicians with useful information regarding the relative risk of infections and lymphopenia which may not be captured by traditional ALC measurement.

Acknowledgements

We would like to thank all the patients and staff of the Fraser Health Multiple Sclerosis Clinic for their support of this research. In particular, thank you to Tatstsyana Kadach for assisting with administration and Dr. Marketa Van den Elzen for editing the manuscript.

Conflict of Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: MEB has received honoraria and participated in consultant advisory board for Novartis. PB reports no disclosures. JN has received honoraria and participated in nursing advisory boards for Biogen Idec, Sanofi-Genzyme, EMD Serono, Hoffman-La-Roche, Novartis, Pendopharm. AK has received honoraria and participated in nursing advisory boards for Biogen Idec, Genzyme, Merck Serono, Novartis, Pendopharm. GV has received research/educational support or honoraria and participated in medical advisory boards for Actelion, Alexioon, BayerHealthcare, Biogen Idec, Celgene (BMS), Genzyme, MedDay, Merck Serono, Novartis, Hoffman La-Roche, Pendopharm, Sanofi-Aventis.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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Supplemental material

Supplemental material for this article is available online.

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