Risk Factors for Lymphopenia in Patients with Relapsing Remitting Multiple Sclerosis Treated with Dimethyl Fumarate

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BACKGROUND

Dimethyl fumarate (DMF) has an associated risk of lymphopenia in relapsing remitting multiple sclerosis (RRMS) patients, affecting primarily CD8+ T-lymphocytes. These lymphocytes may play an important role in the containment of JC virus, the etiologic agent of progressive multifocal leukoencephalopathy (PML). Identifying patients who are at risk for lymphopenia may allow better determination of MS patients to treat with DMF and of appropriate discontinuation of DMF for prevention of PML. Other studies showed lymphopenia occurs more readily in patients previously treated with natalizumab, with advanced age, and with low baseline ALC. We examined additional risk factors or exposures that influence risk of lymphopenia such as carbamazepine, smoking, steroids, opiates, and vitamin D.

OBJECTIVES

1. To identify risk factors for DMF-induced lymphopenia.
2. To characterize the impact of DMF on T lymphocyte subsets in MS patients.

METHODS

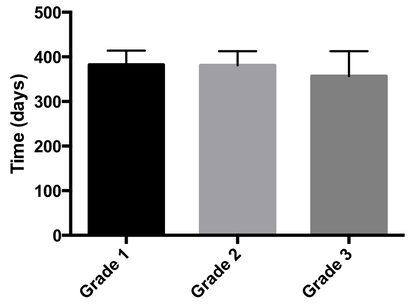
- We performed a retrospective analysis of 196 DMF-treated MS patients at BIDMC since 2013.
- We captured ethnic background, prior medication history, complete blood counts, vitamin D levels and T lymphocyte subsets.
- Possible lymphopenia risk factors examined included age, BMI (Body Mass Index), baseline ALC (Absolute Lymphocyte Count), prior natalizumab exposure, vitamin D levels, and exposure to carbamazepine, opiates, tobacco or steroids while on DMF. Vitamin D levels were corrected for seasonal variation.
- Lymphopenia was defined as grade 1: absolute lymphocytes count (ALC) 800-999/ul; grade 2: ALC 500–799/ul; grade 3: ALC 200-499/ul and grade 4: ALC <200/ul.
- We calculated the cumulative incidence of lymphopenia using standard Kaplan-Meier analysis.
- Using lymphocyte nadir data, we utilized Fisher exact test model to determine which variables were associated with the development of grade 1-3 or grade 2-3 lymphopenia.
- We conducted paired analysis for CD4 and CD8 counts at baseline and nadir using t tests.

RESULTS

Table 1: Demographic data for DMF-treated patient cohort and for lymphopenic patients.

Variable n (%)	Cohort	Grade 1-3	Grade 2-3	Grade 3
Totals	196 (100)	75 (38)	42 (21)	15(8)
Age				
≥40	137(70)	59(78)	35(83)	12 (80)
<40	59(30)	16(22)	7(17)	3 (20)
Gender				
Male	59(29)	20(26)	10(23)	2 (13)
Female	138(71)	55(74)	32(77)	13 (87)
Race				
White	171(86)	68(90)	41(97)	15 (100)
Black	26(14)	7(10)	1(3)	0 (0)
BMI				
>30	50(26)	16(21)	8(19)	4(27)
Vitamin D				
>30ng/ml	137(69)	59(78)	35(83)	12(80)
Exposure to:				
Natalizumab	31(15)	13(17)	8(19)	1(7)
Tobacco	29(14)	7(9)	5(11)	1(7)
Steroids	27(13)	13(17)	8(19)	2(13)
Carbamazepine	21(10)	10(13)	6(14)	1(7)
Opiates	11(5)	4(5)	3(7)	2(13)

Figure 1: Average time to reaching grade 1, grade 2 or grade 3 lymphopenia. There was no significant difference by Kruskal-Wallis testing. This suggests individuals have quite different risks to developing lymphopenia.

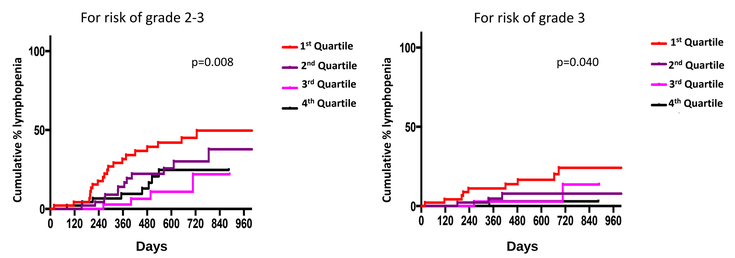


-MS patients were on DMF monotherapy for a median of 17 months.

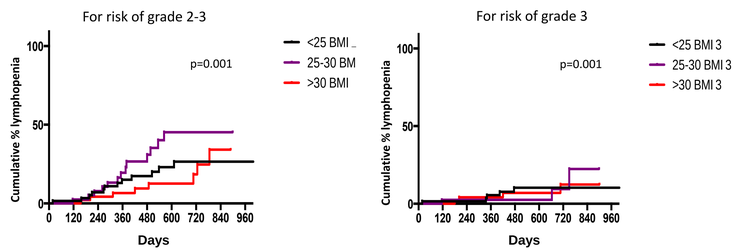
Figure 2: We established the nadir lymphocyte count for each patient and classified each depending on grade. Kaplan Meier analyses were used to model the cumulative incidence of DMF-induced grade 3 or combined grade 2-3 lymphopenia. P values represent the log rank test for differences among groups. (ALC: absolute lymphocyte count)

- Baseline ALC

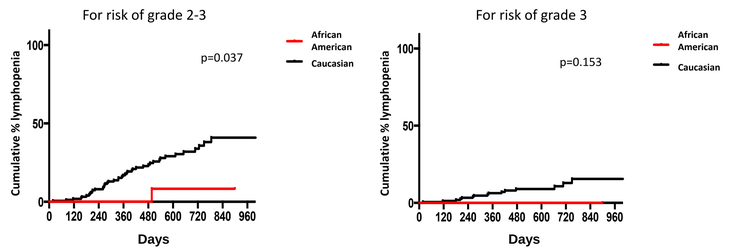
ALC Range:
Quartile 1: 608-1468
Quartile 2: 1469-1845
Quartile 3: 1846-2451
Quartile 4: 2452-4972



- BMI

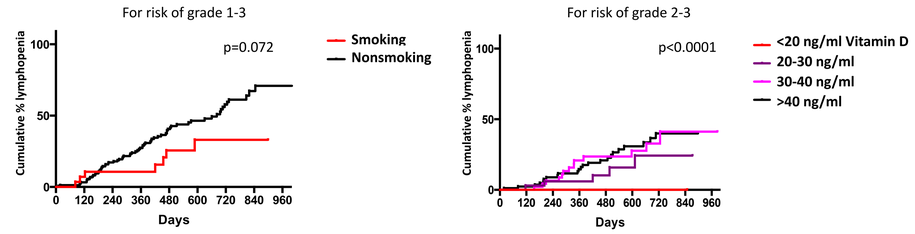


- Race



-Low baseline ALC, BMI 25-30, and white race were significantly associated with differences in cumulative risk of lymphopenia.

Figure 3. There was a trend for significance with smoking at reduced risk of developing grade 1-3 lymphopenia and with high average Vit D at higher risk of lymphopenia grade 2-3



-Gender, prior exposure to natalizumab, steroids, carbamazepine were NOT associated with cumulative risk of lymphopenia. These results differ from previous studies that showed natalizumab affects DMF-related lymphopenia.

Table 2: In subgroup analysis of Kaplan Meier data, we found significant higher cumulative risk of lymphopenia with BMI 25-30 versus BMI >30 and with lowest baseline ALC quartile 1 versus highest quartile 4. At this time, studies vitamin D levels did not reach significance by subgroup analysis with p values at 0.1

Grade lymphopenia	Variable	HR	95% CI	P value (log rank)
Grade 2-3	BMI 25-30 vs >30	2.7	1.1-6.7	**0.02**
Grade 2-3	ALC quartile 1 vs 4	2.4	1.2-5.1	**0.02**
Grade 3	ALC quartile 1 vs 4	4.6	1.3-16.0	**0.02**

Table 3: As another approach we examined risk of whether lymphopenia occurred at any time during DMF treatment. We established the nadir lymphocyte count for each patient and classified each depending on grade. We then tested for significance using Fisher exact test.

Grade Lymphopenia	Age ≥40	Gender	Race	Natalizumab	Smoking	Steroids	Carbamazepine	Opiates	BMI 25-30	Vit D > 30
Grade 1-3	**p= 0.03**	NS	NS	NS	NS	NS	NS	NS	NS	**p= 0.03**
Grade 2-3	**p= 0.03**	NS	**p= 0.01**	NS	NS	NS	NS	NS	NS	**p= 0.03**

-Risk of developing lymphopenia grade 1-3 was significantly greater with vitamin D level >30 ng/ml and age ≥40. Risk of developing lymphopenia grade 2-3 was significantly greater with vitamin D level >30 ng/ml, age ≥40 and white race.

-Gender, BMI, prior exposure to natalizumab, steroids, carbamazepine, tobacco and opiates were NOT significantly associated with lymphopenia in this substudy.

-123 patients had baseline lymphocyte subsets and at nadir lymphocyte count. Compared to baseline levels, CD8+ T-cells were more reduced than CD4+ T-cell cells (67% versus 39% in patients with lymphopenia, p<0.0007) and CD4/CD8 ratio increased from 2.3 to 2.99 in patients with lymphopenia, p<0.0009.

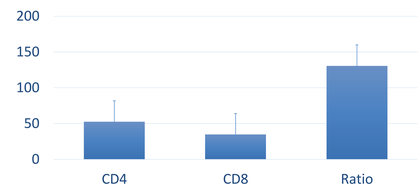


Figure 4. Change in CD4, CD8 and CD4/CD8 ratio in DMF treated patients from pretreatment to nadir. Median days to nadir was 482 days in this substudy.

CONCLUSIONS

1. Grade 2-3 lymphopenia occurs in 21% and grade 3 lymphopenia occurs in 8% of DMF-treated MS patients.
2. Patients with lowest quartile baseline ALC, with BMI 25 to 30, who were older than 40 years old, or with white race had a significantly higher incidence of lymphopenia.
3. Increased vigilance in lymphocyte monitoring is particularly warranted in patients with these characteristics.
4. Lymphopenia in DMF-treated MS reduces CD8+ more than CD4+ T-cells.
5. Higher vitamin D levels above 30ng/ml appear to increase risk of DMF related lymphopenia.
6. If DMF-induced lymphopenia occurs, vitamin D dosing may need to be reduced to prevent further reductions in lymphocytes.