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Risk factors for fingolimod-induced lymphopenia in multiple sclerosis

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Abstract

Background: Lymphopenia is a well-known adverse event of fingolimod, a disease-modifying drug for multiple sclerosis (MS).

Objectives: The objective of this paper is to investigate risk factors for predicting fingolimod-induced lymphopenia in MS by frequent hematological monitoring.

Methods: We retrospectively reviewed data of fingolimod-treated MS patients. Data assessed were sex, age, disease duration, medication history, body mass index, all attacks, Kurtzke's Expanded Disability Status Scale score, and absolute lymphocyte count (ALC) within two days before initiating fingolimod (baseline), on the day after first administration (day 2), and at least every other month after initiating fingolimod therapy.

Results: Of 41 MS patients, marked lymphopenia (ALC $<200/\mu l$) was confirmed in 12 patients (lymphopenia group) within one year. A significantly more frequent history of treatment with any interferon-beta and lower median baseline ALC was observed in the lymphopenia group than in the non-lymphopenia group ($n = 29$) (91.7% vs. 44.8%; $p = 0.006$ and 1469/μl vs. 1879/μl; $p = 0.005$). An ALC of $\leq 952/\mu l$ on day 2 was the most responsible risk factor for predicting marked lymphopenia (sensitivity, 92%; specificity, 76%; area under the curve, 0.823; $p < 0.001$).

Conclusions: Low baseline ALC and treatment history with any interferon-beta were risk factors for fingolimod-induced lymphopenia, possibly predicted from ALC on day 2.

Keywords: Fingolimod, multiple sclerosis, lymphopenia, risk factors, prediction

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Introduction

Fingolimod, a sphingosine-1-phosphate receptor (S1PR) modulator, reduces the recirculation of autoaggressive lymphocytes and is approved as a once-daily oral therapy (dose, 0.5 mg) for multiple sclerosis (MS) in many countries.[1,2] Lymphopenia is a well-known adverse event that affects continuous drug administration because the Summary of Product Characteristics (SPC) in Europe states that if the absolute lymphocyte count (ALC) is confirmed to be $<200/\mu l$, treatment should be interrupted until recovery.[3–6] Patients with a low baseline lymphocyte count and women with a low body mass index (BMI) were suggested to have a risk of lymphopenia in a German and Swedish cohort.[7] However, no studies were based on the data of frequent laboratory tests. Here we aimed to investigate the risk factors for predicting fingolimod-induced lymphopenia in patients with MS by frequent hematological monitoring.

Patients and methods

Patients

First, 70 patients with MS who were treated with fingolimod at Chiba University Hospital until May 2017 were retrospectively recruited. They fulfilled the McDonald criteria proposed in 2010.[8]

Patients who were treated with other S1PR regulators before initiating fingolimod therapy ($n = 3$) and

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those who were followed for <1 year after initiating fingolimod therapy ($n = 18$) were excluded. Eight patients who did not undergo hematological monitoring at any of the following time points were also excluded: within two days before initiating fingolimod therapy (baseline), on the day after the first administration (day 2) of fingolimod therapy, and at least every other month after initiating fingolimod therapy.

Grades of lymphopenia were defined according to the Common Terminology Criteria for Adverse Events (CTCAE v4.0): grade 1 (ALC of 800/μl to the lower limit of normal), grade 2 (ALC 500–799/μl), grade 3 (ALC 200–499/μl) and grade 4 (ALC <200/μl).[9] Patients who exhibited grade 4 lymphopenia within one year after initiating fingolimod were categorized as the lymphopenia group, whereas the remaining patients were categorized as the non-lymphopenia group.

Abnormal liver function tests were defined as any liver enzymes (aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase) were ≥3-fold of the upper limit of normal at two different laboratory examinations.

All patients were originally treated with fingolimod at a dose of 0.5 mg daily; however, 18 patients were switched to intermittent drug holiday therapies such as one drug holiday per week, two holidays per week, or every other day administration.[10] The reasons for drug holiday were the presence of lymphopenia only (grade 4, $n = 9$; grade 3, $n = 3$), abnormal liver function test results only ($n = 3$), and both grade 4 lymphopenia and abnormal liver function test results ($n = 3$).

Informed consent was provided by all patients for participation in this study.

Evaluation points and definitions

We reviewed the patients' data such as sex, age, disease duration, clinical course, medication history, current smoking habits, history of all attacks, weight, BMI, body surface area (BSA) using the Du Bois method, and laboratory tests, including creatinine clearance (CrCl), using the Cockcroft–Gault equation at the initiation of fingolimod therapy.

Moreover, Kurtzke's Expanded Disability Status Scale (EDSS)[11] score and hematological monitoring data, including ALC and liver function test results, were assessed at the following time points: within two days before initiating fingolimod therapy (baseline), on the day after the first administration of fingolimod therapy (day 2), and at least every other month after initiating fingolimod therapy.

Attacks were defined as new or reactivated neurological symptoms caused by an acute inflammatory demyelinating event in the central nervous system, with a duration of at least 24 hours in the absence of fever or infection, and they occurred at least 30 days after the preceding episode.[8]

Statistical analysis

Statistical comparisons between the two groups were performed using the Mann–Whitney U test or Wilcoxon signed-rank test. Fisher's exact test was performed to compare dichotomous variables. Univariable and multivariable logistic regression analysis models were used to estimate the risk factors associated with grade 4 lymphopenia. The receiver operating characteristic curve was used to identify the selection of cut-off points for regression analysis, and area under the curve (AUC) was used for model comparison to identify the responsible predictor for grade 4 lymphopenia; Kaplan–Meier survival plot was used to test the efficacy of early predictors.

Differences of p values of <0.05 were considered to be statistically significant. All statistical analyses were performed using the JMP® 12 software (SAS Institute Inc, Cary, NC, USA).

Results

In total, 41 patients fulfilled the inclusion criteria. The mean number of hematological monitoring within one year after initiating fingolimod therapy was 14.2 (range, 11–30). The baseline characteristics of the patients in the lymphopenia group ($n = 12$) and non-lymphopenia group ($n = 29$) are summarized in Table 1. The distributions of sex, age, disease duration, clinical patterns, smoking habits, annualized relapse rates (ARRs), EDSS scores, weight, BMI, BSA, and CrCl were not significantly different between the two groups.

The history of treatment with any interferon-beta (IFN-β) was significantly more frequent in the lymphopenia group than in the non-lymphopenia group (91.7% vs. 44.8%; $p = 0.006$). The median (interquartile range; IQR) ALC at baseline was 1469.0/μl (413.5/μl) in the lymphopenia group and 1879.0/μl (796.0/μl) in the non-lymphopenia group ($p = 0.005$), and the time series charts of ALC in

Table 1. Comparison of baseline[a] characteristics in the lymphopenia and non-lymphopenia group.

Characteristic	Lymphopenia group ($n = 12$)	Non-lymphopenia group ($n = 29$)	p value
Clinical pattern of MS, RR:SP	11:1	27:2	0.657
Female, *n* (%)	11 (91.7)	19 (65.5)	0.087
Age, median (IQR)	40.5 (9.3)	35.0 (15.5)	0.456
Disease duration in years, median (IQR)	13.2 (14.4)	8.2 (13.9)	0.218
ARRs within previous two years, median (IQR)	1.25 (2.75)	1.00 (2.00)	0.738
EDSS score, median (IQR)	3.75 (3.00)	3.00 (4.00)	0.394
Weight, kg, median (IQR)	54.7 (5.3)	54.3 (16.2)	0.785
BMI, kg/m^2, median (IQR)	20.4 (3.4)	21.3 (3.4)	0.456
BSA, m^2, median (IQR)	1.57 (0.08)	1.56 (0.26)	0.752
CrCl, ml/min, median (IQR)	121.6 (26.2)	107.2 (27.8)	0.390
Current smoker, *n* (%)	5 (41.7)	10 (34.5)	0.464
Treatment history, *n* (%)	11 (91.7)	13 (44.8)	0.006
Any INF-β, *n* (%)	11 (91.7)	13 (44.8)	0.006
Natalizumab, *n* (%)	1 (8.3)	0 (0)	0.300
Absolute lymphocyte count, /μl, median (IQR)	1469.0 (413.5)	1879.0 (796.0)	0.005

ARRs: annualized relapse rates; BMI: body mass index; BSA: body surface area; CrCl: creatinine clearance; EDSS: Expanded Disability Status Scale; IFN-β: interferon-beta; IQR: interquartile range; MS: multiple sclerosis; RR: relapsing–remitting; SP: secondary progressive.
[a]At the initiation of fingolimod.

the lymphopenia group were continuously less than those in the non-lymphopenia group until one year after initiating fingolimod treatment (Figure 1).

The characteristics of the patients at last follow-up are summarized in Table 2. The total administration duration, ARRs, EDSS score, and proportion of patients who exhibited abnormal liver function test results were not significantly different between the two groups. In all patients, the elevations of liver enzyme levels were asymptomatic. The median (IQR) decline rate of ALC, which was obtained by dividing the lowest ALC within one year after initiating fingolimod by the baseline ALC was 11.1% (5.8%) in the lymphopenia group and 17.8% (10.9%) in the non-lymphopenia group ($p < 0.001$). The number of patients treated with intermittent drug holiday therapy was significantly higher in the lymphopenia group than in the non-lymphopenia group (75.0% vs. 31.0%; $p = 0.012$). The median (IQR) duration of intermittent drug holiday therapy was 1.9 (2.9) years in the lymphopenia group and 0.0 (0.6) years in the non-lymphopenia group ($p = 0.006$). Administration frequency at last follow-up was significantly lower in the lymphopenia group than in the non-lymphopenia group (4.0 days per week vs. 7.0 days per week; $p = 0.001$).

The logistic regression model showed an increased risk with ALC of $\leq$1675/μl at baseline both in the univariable and multivariable regression analyses (Table 3). Furthermore, patients who were previously treated with any IFN-β had a higher risk of grade 4 lymphopenia. Female sex and BMI of $\leq$19.8 kg/m^2 had no statistical effect on the risk of grade 4 lymphopenia.

Figure 2 shows that compared with the baseline ALC, an ALC of $\leq$952/μl on day 2 was a more responsible risk factor for predicting grade 4 lymphopenia within one year, with a sensitivity of 92% and specificity of 76% (AUC, 0.823; $p < 0.001$). Furthermore, this early predictor was effective for distinguishing grade 4 lymphopenia at the last follow-up (Figure 3).

Discussion

This study showed that a low ALC at baseline and a treatment history of any IFN-β were associated with grade 4 lymphopenia, and an ALC of $\leq$952/μl on the day after first administration (day 2) was an early predictor.

Fingolimod has a long half-life of approximately six to nine days, and it takes >4 weeks to reach a steady-state concentration in the blood.[4,12] Grade 4 lymphopenia

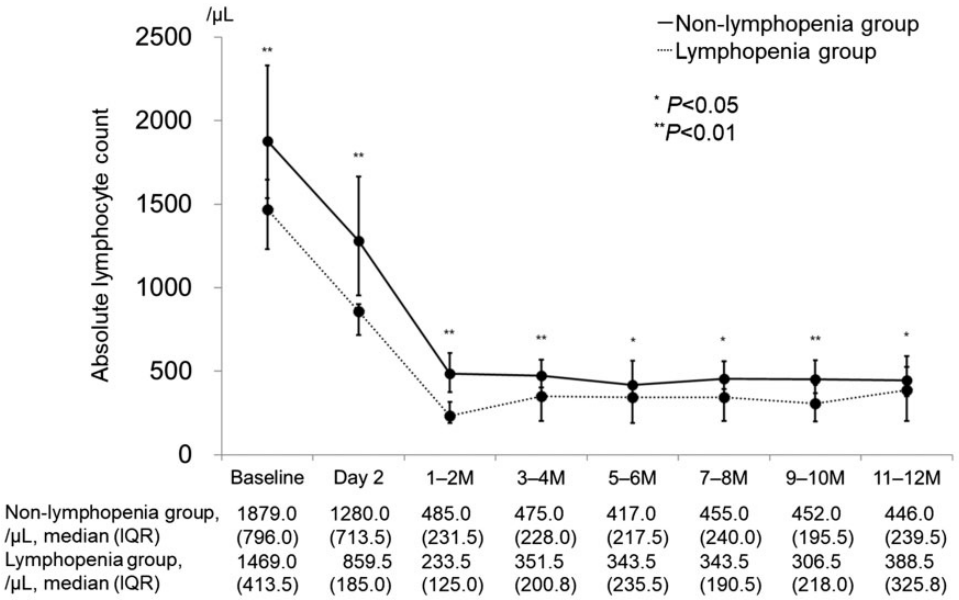

Figure 1. Longitudinal change of absolute lymphocyte count in lymphopenia group ($n = 12$) and non-lymphopenia group ($n = 29$). Baseline is within two days before initiating fingolimod treatment, and day 2 is the day after the first administration.
IQR: interquartile range; M: month.

Table 2. Comparison of characteristics at last follow-up after the initiation of fingolimod in lymphopenia and non-lymphopenia group.

Characteristic	Lymphopenia group ($n = 12$)	Non-lymphopenia group ($n = 29$)	p value
Total administration duration in years, median (IQR)	2.3 (2.0)	3.6 (2.7)	0.288
ARRs[a], median (IQR)	0.27 (1.20)	0 (0.26)	0.055
EDSS score, median (IQR)	4.25 (3.75)	3.50 (4.50)	0.574
Abnormal liver function tests[b], n (%)	3 (25.0)	8 (27.6)	0.595
Decline rate of absolute lymphocyte count[c], %, median (IQR)	11.1 (5.8)	17.8 (10.9)	<0.001
Patients with IDH therapy, n (%)	9 (75.0)	9 (31.0)	0.012
Administration duration of IDH therapy in years, median (IQR)	1.9 (2.9)	0.0 (0.6)	0.006
Administration frequency, days per week, median (IQR)	4.0 (3.8)	7.0 (1.0)	0.001

ARRs: annualized relapse rates; EDSS: Expanded Disability Status Scale; IDH: intermittent drug holiday; IQR: interquartile range.
[a]ARRs were obtained by dividing the total number of relapses by total duration after the initiation of fingolimod.
[b]Abnormal liver function tests were defined as any liver enzymes (aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase) were ≥3-fold of the upper limit of normal at two different laboratory tests.
[c]Decline rate of absolute lymphocyte count was obtained by dividing the lowest absolute lymphocyte count within one year after initiating fingolimod by the baseline absolute lymphocyte count.

during fingolimod therapy is a common adverse event, occurring in 15%–20% of patients.[7,13] Previous studies have reported that the decrease of nadir ALC was correlated with the dosage, and patients with a low initial lymphocyte count (<1600/μl) and females with low BMI (<18.5 kg/m^2) were at a higher risk of developing lymphopenia.[3,7]

In our study, grade 4 lymphopenia within one year was observed in 29.3% (12/41) patients, which was much higher than previously reported values. The mean BMI of the 41 patients in this study was 22.0 kg/m^2 (vs. 24.3–25.4 kg/m^2 in a previous study).[7] Given that the lymphocyte count is affected by BMI,[14] the higher proportion of patients with grade 4 lymphopenia in

Table 3. Logistic regression model: Risk factors associated with grade 4 lymphopenia (ALC <200/μl).

Risk factors		Odds ratio (95% confidence interval)	*p* value
Sex, female vs. male		5.79 (0.92–113.52)	0.064
	Adjusted[a]	6.60 (0.46–254.67)	0.171
BMI ≤19.8kg/m^2[b], yes vs. no		2.63 (0.65–10.97)	0.174
	Adjusted[a]	6.83 (0.83–181.09)	0.077
History of any IFN-β treatment, yes vs. no		13.53 (2.20–264.18)	0.003
	Adjusted[a]	23.50 (1.77–1250.57)	0.013
	Adjusted[c]	10.18 (1.40–212.26)	0.020
ALC of ≤1675/μL at baseline[b], yes vs. no		18.00 (2.91–352.56)	<0.001
	Adjusted[a]	38.52 (3.48–1803.71)	0.001
	Adjusted[c]	14.15 (2.04–289.97)	0.005

ALC: absolute lymphocyte count; BMI: body mass index; IFN-β: interferon-beta.
[a]Adjusted for sex, BMI ≤19.8kg/m^2 or more, medication history, ALC of ≤1675/μl or more at baseline.
[b]The optimal value was inferred by receiver operating characteristic curve.
[c]Adjusted for medication history, ALC of ≤1675/μl or more at baseline.

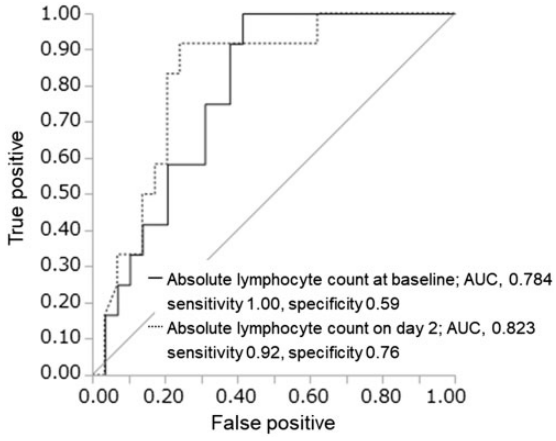

Figure 2. Prediction of grade 4 lymphopenia (absolute lymphocyte count <200/μl) within one year by lymphocyte count at baseline (within two days before initiating fingolimod treatment) and on day 2 (the day after the first administration).
AUC: area under the curve.

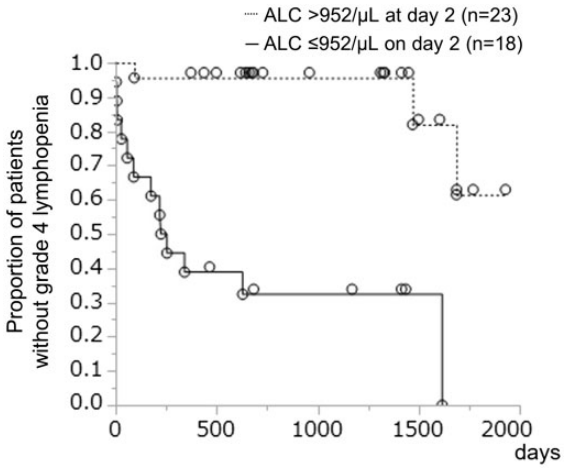

Figure 3. Days to fulfill the definition of grade 4 lymphopenia (absolute lymphocyte count <200/μl). Kaplan–Meier survival plot tested in two groups that were divided by absolute lymphocyte count on day 2 (the day after the first administration): Absolute lymphocyte count was 952/μl or less ($n = 18$) and more than 952/μl ($n = 23$). In the group with absolute lymphocyte count of 952/μl or less, the median (95% confidence interval) days to fulfill grade 4 lymphopenia were 239 days (57–1617 days, $p < 0.001$).
ALC: absolute lymphocyte count.

this study compared with those in previous studies could be attributed to lower BMI in this population. However, in this population, a low ALC at baseline was the main risk factor for grade 4 lymphopenia, and female sex and low BMI showed the same trends as those reported in a previous study.

The IFN-β family influences the production of cytokines by lymphocytes and some adverse events such as leukopenia and lymphopenia,[15] which are considered to be related to myelosuppressive activities.[16]

Although ARRs before fingolimod administration were similar between the lymphopenia and non-lymphopenia groups, ARRs after fingolimod therapy

tended to be slightly higher in the lymphopenia group. This finding might be because of the higher rates of patients treated with intermittent drug holiday therapy in the lymphopenia group. There is a possibility that the change from conventional therapy to intermittent drug holiday has a risk of rebound effects,[17] and it was reported that the efficacy of an alternate-day fingolimod administration was not effective enough to inhibit disease activities, though dose-reduction therapy was safe overall and well tolerated with some efficacy.[12,18,19] Our study was a retrospective one, and we had not defined the number of lymphocyte counts to start intermittent drug holiday therapy. However, we started intermittent drug holiday therapy when an ALC was confirmed to be <200/μl or when the next ALC was supposed to be <200/μl judging from the ALC and the speed of decrease of ALCs.

In a phase III fingolimod study, the rate of infections was not correlated with the lymphocyte count.[13] However, whether fingolimod-induced lymphopenia affects progressive multifocal leukoencephalopathy and other opportunistic infections remains unclear.[20] As described in the SPC, fingolimod-induced lymphopenia should be detected as soon as possible.[6] The predictor identified in this study may aid in the early detection of lymphopenia, resulting in the prevention of adverse events, including infections.

The limitations of this study include its retrospective design and small sample size; furthermore, frequent hematological monitoring was performed only for one year. The different percentages of patients who were treated with intermittent drug holiday therapy, and different drug administration durations of intermittent drug holiday therapy and frequencies between lymphopenia and non-lymphopenia groups, might have affected the comparisons of the last follow-up characteristics. Moreover, the lack of uniformity in the lymphopenia group due to the retrospective design of our study including concomitant disorder such as liver dysfunction might also have affected the results. Accordingly, a large randomized controlled trial should be performed to confirm our findings.

In conclusion, a low baseline ALC and a treatment history with any IFN-β were risk factors for fingolimod-induced lymphopenia, and patients with an ALC of ≤952/μl on the day after first administration were more likely to develop lymphopenia after fingolimod treatment; such patients may need to be carefully monitored and prepared for the appearance of adverse infectious events.

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Conflicts of interest

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