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Lymphocyte count in peripheral blood is not associated with the level of clinical response to treatment with fingolimod

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ABSTRACT

Background: Fingolimod is an efficient and safe drug for treating relapsing-remitting multiple sclerosis (RRMS). *In vivo*, fingolimod is phosphorylated and binds to "sphingosine-1-phosphate"(S1P) receptors that are expressed in a wide range of cells, including lymphocytes. Under the effect of fingolimod, lymphocytes are retained in lymphoid tissues through the regulation of $S1P_1$ receptors. The aim of the present study was to assess whether the degree of lymphopenia was correlated to the positive treatment response of RRMS patients with fingolimod.
Methods: Data was sourced from the MSBase Registry. Patients were divided into two groups, according to the lymphocyte count on peripheral blood examination. Annualized Relapse Rate (ARR), time to first relapse and time to six-month confirmed disability progression were compared between groups.
Results: Group one consisted of 202 patients who reached 750 lymphocytes/mm^3 during treatment while the comparison group two included 101 patients who never reached less than 1000 lymphocytes/mm^3 in peripheral blood during the observation period. There were no differences between groups in ARR, time to first relapse or time to six-month confirmed disability progression.
Conclusion: The degree of lymphopenia in peripheral blood was not associated to the positive treatment response of fingolimod in RRMS patients.

1. Introduction

Fingolimod is a relatively new, efficient and safe drug for treating relapsing-remitting multiple sclerosis (RRMS) (Jeffery et al., 2016). *In vivo*, fingolimod is phosphorylated and resembles naturally occurring "sphingosine-1-phosphate" (S1P). S1P binds to receptors that are expressed in a wide range of cells involved in many biological processes relevant to RRMS. The main mechanism of action of fingolimod in RRMS is the retention of lymphocytes in lymphoid tissues through the regulation of $S1P_1$ receptors expressed on lymphocytes (Chun and

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Table 1
Comparison of baseline characteristics in the 2:1 propensity score matched sample.

Baseline factor	< 0.75 ever group (n = 202)	> 1.0 always group (n = 101)	p (signed-ranks)	Standardised difference^
Female sex - n (%)	139 (68.8)	66 (65.4)	0.5430	0.074
Age (years) - median (IQR)	40.2 (9.9)	40.3 (9.1)	0.9285	0.003
EDSS - median (IQR)	2.5 (1.5, 4.5)	2.5 (1.5, 4)	0.8356	0.025
Disease duration (years) - median (IQR)	10.2 (7.9)	10.6 (7.7)	0.5952	−0.045
Number of prior DMD treatment starts - median (IQR)	2 (1, 3)	2 (1, 3)	0.7944	−0.059
Proportion of disease duration on DMD treatment - mean (SD)	0.50 (0.30)	0.50 (0.31)	0.9534	0.020
Total relapse onsets last 12 months – mean(SD)	0.9 (1.0)	0.9 (1.1)	0.929	−0.048
Total relapse onsets last 24 months – mean(SD)	1.6 (1.3)	1.7 (1.4)	0.7930	−0.048
*Additional matched on country				
^Standardised difference between −0.15 & +0.15 considered acceptable balance				

Hartung, 2010). The antagonism of the $S1P_1$ receptor's function leads to a decrease in lymphocyte egress from lymphoid tissues into the circulation resulting in peripheral blood lymphopenia, typical of treatment with fingolimod (Chun and Hartung, 2010; Subei and Cohen, 2015). In order to understand whether the degree of fingolimod-associated lymphopenia might be correlated to the effect of this drug in RRMS, the present study was carried out. It analysed, in a real-world setting, whether the level of decrement in the lymphocyte count in peripheral blood could positively influence patients' response to treatment.

2. Method

2.1. Data collection

Data were obtained from the MSBase Registry, established in 2004 to collect disease-related information from consenting patients attending MS clinics. The registry's member centers, almost exclusively large academic MS centers, follow a defined minimum dataset protocol to prospectively collate outcomes data using an internet-based, physician owned and operated system. In the present study, participants were invited to join a substudy on the potential association of peripheral lymphocyte counting and efficacy of fingolimod. At each contributing center the project had Human Research Ethics Committee approval or exemption, in accordance to MSBase rules and regulations.

2.2. Inclusion criteria

Patients in the MSBase registry recording complete relapse, progression and clinical data across a minimum of two visits since initiating fingolimod were included. In addition, patients were required to have at least two blood tests with full lymphocyte counts available for analyses.

2.3. Patient groups

Patients were divided into two groups: those who reached less than 750 lymphocytes/mm^3 in peripheral blood at any given time on fingolimod (< 750-ever group); and a propensity matched group who never had recorded less than 1000 lymphocytes/mm^3 in peripheral blood (< 1000-never group) on fingolimod. Alternate lymphocyte groups based on different cut-points of lymphocyte count were trialed, however these were rejected on the grounds of insufficient event number and power.

2.4. Statistical analyses

Categorical variables were summarised using frequency and percentage. Continuous variables were summarised using mean and standard deviation (SD) or median and inter-quartile range (IQR) as appropriate. Patients in the higher lymphocyte group were propensity score matched on a 1:2 basis with comparable patients in the lower lymphocyte count group. The propensity score was derived using a logistic regression model defined as a function of the following key prognostic correlates of our end-points at baseline: age, sex, disease duration, disability according to the expanded disability scale score (EDSS), prior treatment exposure and pre-baseline relapse activity. Balance between study groups in the matched sample was assessed through the derivation of standardized differences and either a McNemar chi-square test or signed-ranks tests as appropriate. Annualized Relapse Rate (ARR) was compared between groups using a paired signed-ranks test. Time to first relapse and time to six-month confirmed disability progression were compared using a marginal Cox model. Hazard proportionality was assessed via analysis of scaled Schoenfeld residuals. Six-month confirmed disability progression was defined as ≥6-month confirmed increases of ≥0.5-point for patients with a baseline EDSS score > 5.5, ≥1.0-point for those with a baseline EDSS score between 1.0 and 5.5, inclusive, and ≥1.5-point for those with a baseline EDSS score of 0. EDSS scores recorded within 30 days after the onset of a relapse were excluded. As a sensitivity analysis of the primary modelling, *continuous* lymphocyte counts (modelled as both non-transformed and log-transformed variables) were analysed for association with ARR using Generalized Estimating Equations (GEE). For all analyses $p < 0.05$ was considered significant. All analyses were undertaken in Stata version 14 (StataCorp, College Station, Texas).

3. Results

Of the 908 " < 1000 never" group patients and 101 " < 750 ever" group patients satisfying the inclusion criteria, a total of 202 patients in the < 750-ever group were successfully propensity matched on a 2:1 basis to 101 patients in the < 1000-never group. The matched sample was well balanced for all baseline covariates across the two groups with all absolute standardized differences less than 10% (Table 1). In addition, all pair-wise signed-rank tests were non-significant. Mean (SD) follow-up time for the < 750 and < 1000-never groups were 2.31 (1.28) and 1.91 (1.18) years respectively. The median (IQR) time to lymphocyte nadir in the propensity-score matched sample from the date of fingolimod initiation was 5.0 months (1.8, 10.3).

3.1. Relapse

There was no difference in ARR between groups ($p = 0.2967$) with the < 750 ever group recording a mean (SD) ARR of 0.34 (0.84) compared with 0.52 (0.98) in the < 1000 never group. Of the < 750-ever group, 36.1% experienced at least one relapse event compared to 38.6% of the matched < 1000-never group. There was no difference between lymphocyte groups in time to first relapse (HR 0.79; 95% CI 0.54, 1.15; reference = < 1000 never) (Fig. 1). Consistent with the primary analysis, the continuous GEE modelling suggests no association between lymphocyte count and outcome ($p = 0.333$ if one-unit increase in lymphocyte count across the observation period was to be associated with a 0.001 unit increase in ARR).