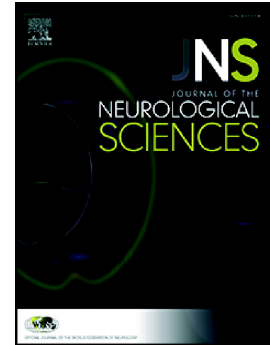# Accepted Manuscript

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Abstract

Fingolimod exerts its therapeutic effect in multiple sclerosis by modulating sphingosine-1P receptors which are expressed in the heart mediating fingolimod first dose effects. Understanding potential interactions of baseline characteristics and autonomic profile with fingolimod first dose effects may add novel safety information and help explain cases requiring extension of the 6-hour ECG monitoring period. We aimed at characterizing the patient population treated with the first dose of fingolimod in clinical practice in an observational, multicenter, prospective 6-hours (up to 24) study. ECG was recorded for 15 min before first fingolimod administration and for 6 hours after. Heart rate (HR) and HR variability in the frequency domain were derived from ECG traces. Out of the 625 enrolled patients, 580 (92.8%) were discharged at the sixth hour after fingolimod first dose; 45 (7.2%) required monitoring extension. Data confirm the well characterized cardiovascular fingolimod profile upon treatment initiation. Ten (1.6%) patients showed an atrioventricular block, all asymptomatic and self-resolving. Normalized spectral power in the High Frequency band (marking vagal modulation) and previous annualized relapse rate were independently correlated with the probability of undergoing extended monitoring. Our results could provide useful information for the stratification and individualized monitoring of MS patients prescribed with fingolimod.

Keywords

1. Introduction

Fingolimod is an immunomodulatory drug approved in Europe as second-line treatment for relapsing-remitting multiple sclerosis (RRMS). It exerts its therapeutic effects in MS by modulating sphingosine-1-phosphate (S1P) receptors, and the key mechanism for these effects is its action on S1P receptors type 1 (S1P1) expressed on lymphocytes [1]. These receptors are also expressed on cardiac tissues [2, 3] and cause transitory vagomimetic effects resulting in a heart rate (HR) reduction. This decrease may, in some cases, result in bradycardia (HR <50 beats/min) and atrioventricular (AV) blocks, usually first-degree, rarely second-degree Mobitz class I [1]. It has been shown that fingolimod effect on HR depends on the S1P- mediated activation of an inwardly rectifying Gαi-protein-regulated potassium channel (GIRK/IKACh) expressed on atrial myocytes and endothelial cells [2]. However, such effect occurs prevalently upon the first administration, as

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the fingolimod agonistic activity on S1P1 produces a rapid receptor internalization and denaturation. The current EU fingolimod Summary of Product Characteristics specifies that all patients should have a 12-lead ECG and blood pressure measurement performed prior to and 6 hours after the first dose administration of the drug. Furthermore, during this 6-hour period, it is recommended that all patients are monitored for signs and symptoms of bradycardia and for the occurrence of AV blocks by hourly HR and blood pressure measurement and by continuous (real time) ECG monitoring. If the HR at 6 hours is the lowest since the first dose was administered (suggesting that fingolimod maximum pharmacodynamic effect has not been reached yet), monitoring should be extended by at least 2 hours and until heart rate increases again. Additionally, if at the 6th hour post-dosing, the HR is <45 bpm, or the ECG shows new onset of second degree or higher grade AV block or a QTc interval ≥500 msec, extended monitoring should be performed at least overnight or until the findings have resolved. The occurrence at any time of third degree AV block should also lead to extended monitoring (at least overnight monitoring). Overnight extension of the monitoring period needs to be considered in case of post-dose bradyarrhythmia-related symptoms, until the symptoms have resolved.

The preeminent mechanism involved in the fingolimod HR effects is that its intracellular pathway involving G protein activation might converge with the one triggered by M2 receptors activation at the level of the atrial muscarinic-gated potassium channel I_{KACh}, thus resulting in a converging action on sinus node pace maker firing. This hypothesis of a synergy between fingolimod cardiac effects and cardiac vagal modulation can be effectively explored by looking at a consolidated autonomic index like the variability of HR (HRV) [4]. Recent studies demonstrated that fingolimod can modify autonomic function upon administration [5, 6] and a possible correlation between HRV and post-fingolimod first dose events has been postulated with contradictory results [7, 8]. Therefore, we wanted to investigate any potential interaction between the individual autonomic profile and the need of extended monitoring due to HR and impulse conduction alterations. Thus, the main purpose of this study has been to characterize the population for whom any extension of monitoring was necessary after routine first fingolimod dosing, in order to understand whether there are autonomic factors contributing to the observed HR effects.

2. Material and Methods

2.1 Objectives

The study aimed at characterizing the RRMS population for whom a prolongation of monitoring

was necessary after the first dose of fingolimod. We focused on the following parameters:

- demographic variables (gender, age);

- MS disease duration, degree of neurological disability (Expanded Disability Status Scale, EDSS) at inclusion, Annualized Relapse Rate (ARR) in the year before inclusion;

- concomitant use of drugs that could affect HR and/or AV conduction;

- autonomic nervous system balance as assessed by resting spectral HRV;

- any concomitant co-morbidity (ischemic heart disease, cerebrovascular disease, congestive heart failure, history of cardiac arrest, uncontrolled hypertension, severe untreated sleep apnea) and concomitant medications that could potentially be associated to extended monitoring.

The study design allowed also to explore whether a correlation existed between basal vagal tone and the relative maximum percentage reduction in HR during the six hours of monitoring after the first dose of fingolimod.

2.2 Study design

This is an observational, prospective, multicenter study. Study duration was limited to the fingolimod first dose observation period, i.e. 6 hours in the routine cases or up to 24 hours (or, in any case, until resolution of the intervening clinical condition) if an extension of the monitoring period was required. Drug administration was preceded by a continuous ECG recording of at least 15 minute duration and followed by the subsequent clinical and ECG continuous monitoring period of 6-hours. Vital signs were also assessed as per routine clinical practice.

If an extension of the monitoring period was required and patient observation was prolonged, continuous ECG was maintained until patient discharge.

2.3 Inclusion criteria

- Male or female outpatients ≥18 years and diagnosed with RRMS for whom the decision to start treatment with fingolimod had already been taken, based on clinical practice and according to the fingolimod Summary of Product Characteristics and to AIFA criteria for inclusion in the national drug monitoring registry and independently of the decision to enroll the patient into the study.

- a signed (by the patient him/herself or by a legal representative) written informed consent

2.4 Exclusion criteria

- Early and permanent discontinuation of fingolimod in a previous fingolimod trial due to AE or SAE or abnormal laboratory finding (i.e. conditions such as macular edema, hepatic

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enzymes greater than 5 times the upper normality limits, every kind of malignancy) except for pregnancy.

- Any clinical condition that may interfere with the subject's ability to cooperate and comply with study procedures.
- Patient included in an experimental/clinical study at baseline visit.

During the study visit the following patient demographic and baseline characteristics were collected: age, gender and female reproductive history (number of pregnancies, number of sons/daughters), education, race and occupational status. In addition, various MS-related characteristics such as date of MS diagnosis, date of first MS symptoms, eye disease history (e.g. optic neuritis, uveitis), number of relapses in the previous year and history of medications used to treat MS were recorded. Relevant medical history/current medical condition were recorded either before inclusion in the study or at study entry. The medical history took specifically into consideration concomitant and previous diseases possibly associated with extended monitoring such as: diabetes, ophthalmological disease, cerebrovascular disease history of myocardial infarction, congestive heart failure, history of cardiac arrest, uncontrolled hypertension, severe untreated sleep apnea, infections. Concomitant medications to treat MS-related symptoms were also recorded as well as the previous MS treatment. All prior and prior/concomitant medications that can alter HR or AV conduction were recorded in the appropriate eCRF form.

At the end of the monitoring period, a first dose monitoring experience survey was administered to the patients.

2.5 Heart Rate Variability

Patient HRV data were obtained by analysis of electrocardiogram registrations by means of a dedicated software (Heartscope, by AMPS LLC, New York) [9, 10]. Briefly, the time series of consecutive RR intervals (commonly referred to as tachogram) were automatically derived from the ECG traces by first detecting the QRS complex of each cardiac beat and secondly by assessing the apex of each R wave with parabolic interpolation. All the ECG traces and the associated RR time series were carefully reviewed to identify ectopic beats, artifacts or missing beats. In each patient, stationary segments of 250±50 consecutive RR intervals were finally selected for HRV computation with power spectra analysis by linear (autoregressive modeling) approaches [4, 11].

All selected segments and related power spectra were evaluated by two experienced specialized cardiologists in blind conditions to avoid any bias in the final HRV scoring.

Quality control of the correct data workflow was organized by the study Contract Research

Organization (Medidata) to warrant correct matching of the ECG recording with HRV scoring and patient final autonomic labeling (AMPS).

A group of 150 tachograms were selected for double-check blind ECG and HRV scoring. The double assessment was further validated by a third independent cardiologist who confirmed coherence of the two assessments in 100% of cases. A specific aspect of this review process, specifically relevant to the appropriate definition of the bradycardia events (due or not to AV blocks) was that all abnormal RR intervals were electronically and visually reviewed, thus minimizing the risk inherent to potential inappropriate automatic detection.

Frequency domain analysis was used to define HRV. This approach identifies specific oscillations in heart rate that reflect the activity of the two components of the autonomic nervous system cardiac control. Respiration produces periodic oscillations in vagal cardiac activity. These oscillations reflect into heart rate oscillations that have the respiratory frequency (e.g. 16/min or 0.25 Hz). On the other hand, sympathetic activity produces HR oscillation at a lower frequency (see LF below). Very low frequency (VLF) HR oscillations reflect termoregolatory and other hormonal humoral factors. For the present investigation short segment ECG recordings were used and VLF were not sufficiently represented and thus, not used for HRV analyses. VLF power band limit was set to actually properly estimate the lower limit of the LF band. The amplitude of the oscillations is then the real measure of ANS activity and it is quantified by the power comprised in each band.

Thus, the following bands of spectral analysis were calculated [13, 14]:

- VLF (very low frequency, < 0.03 Hz)
- LF (low frequency, 0.03-0.15 Hz)
- HF (high frequency, > 0.15 Hz)

The following HRV parameters from the pre dosing ECG recording were then provided:

- Total spectral power (RR TP)
- Absolute and normalized spectral power in the VLF, LF and HF bands. The Ratio between the absolute spectral power in LF and in HF band (LF/HF) was also computed.

As to the 6-hours continuous ECG recording after the first dose administration, the following parameters were provided:

- Value of the single maximum RR interval
- Value of the maximum average RR interval computed on 8 consecutive RR intervals regarding beats with sinus rhythm.

2.6 Medication of interest

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The medication of interest was, by definition, fingolimod (Gilenya®).

Previous steroid treatment of MS relapses, previous therapies for MS (last year), other relevant therapies that could alter HR or AV conduction administered prior to enrollment or during the observation period, other pharmacological therapies for comorbidities ongoing at baseline were recorded.

2.7 Sample size and statistical analysis

Sample size of 600 evaluable patients was determined on the basis of simulations of achievable precision of the estimate in different conditions (which also included feasibility). In particular, assuming a percentage of 8% of patients who could require extended monitoring, a sample of 600 evaluable patients would provide an association level of 0.50 (0.28-0.90) (expected odds ratio, OR, with 95% confidence interval) between each factor possibly affecting the monitoring extension and the latter condition.

The statistical analysis was done on all evaluable patients who entered the study and received the first dose of fingolimod and for whom data about the decision to stop or extend ECG monitoring beyond 6 hours was available.

Quantitative variables were described by mean, standard deviation, median, first and third quartile, minimum and maximum, while qualitative variables by absolute and relative frequency.

Logistic regression models were summarized by means of odds ratios (OR) and their 95% confidence interval (CI). A multivariate logistic regression model was used, with backward selection method in order to identify the determinant to extended monitoring.

The correlation was assessed by means of scatter plots and Spearman correlation coefficient.

Relative maximum percentage reduction in HR during the 6-hour monitoring after the first dose of fingolimod was computed as follows: RMP = ((pre-dose HR) – min (HR) / pre-dose HR)*100). Pre-dose HR is the pre-dose HR value retrieved from HRV data while min (HR) was the minimum among sitting pulse values measured at each hour during the 6-hour monitoring, as reported by the clinical investigator in the eCRF. Patients with negative values of relative maximum percentage of HR reduction were excluded from this analysis.

2.8 Ethical standards

The study has been approved by the local Ethic Committees of the Centers involved in the study under the coordination of the Ethic Committee of the leading center of Genova (see affiliation Prof. G. Mancardi). The study and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants gave their written

informed consent prior to their inclusion in the study.

3. Results

3.1 Study population and adverse events

Overall, 628 patients were enrolled in the study. Of these, 3 (0.5%) patients were excluded from the analysis due to: date of RRMS diagnosis not available (1 patient), inclusion in an experimental/clinical study at baseline visit (1 patient), and no fingolimod intake. The study population characteristics are detailed in table 1.

Mean time from MS diagnosis was 7.63 ± 6.36 years. Mean age at diagnosis was 30.65 ± 9.55 years. EDSS score at entry was 2.84±1.58 and the mean number of relapses in the last year was 1.13±0.70. The survey collected at the end of the observation time indicated that the monitoring experience was mostly satisfactory (mean score of 8.0 ± 2.0) with a low stress impact by monitoring process (mean score of 4.3 ± 3.0).

During the observation time 27 (4.3%) patients experienced at least one adverse event and 6 (1.0%) patients had a serious adverse event (5 patients experienced AV blocks and one sinus bradycardia); twenty five (4.0%) patients had an adverse event suspected of a causal relationship to fingolimod and 4 (0.6%) had adverse events leading to fingolimod discontinuation (table 2).

Table 1. Demographic data (evaluable patients)

Age at entry (years)		
	N	625
	Mean	38.26
	SD	9.68
	25th percentile	31.00
	Median	38.00
	75th percentile	45.00
	Min	18.00
	Max	66.00
Age classes		
	<= 40 years	363 (58.1%)

	> 40 years	262 (41.9%)
Sex		
	Female	419 (67.0%)
	Male	206 (33.0%)
Race		
	Caucasian	619 (99.04%)
	Asian	4 (0.64%)
	American Indians/Alaska natives	2 (0.32%)
Years from MS diagnosis (mean ± SD)		7.63 ± 6.36
Age at MS diagnosis (mean ± SD)		30.65 ± 9.55
EDSS score at entry (mean ± SD)		2.84 ± 1.58
N of relapses in the last year (mean ± SD)		1.13 ± 0.70

Table 1: detailed summary of study population characteristics (evaluable patients); EDSS = Expanded Disability Status Scale; MS = multiple sclerosis; SD = standard deviation

Table 2. Summary of principal adverse events

System Organ Class	Preferred term	N=625 (%)
N of pts with at least one adverse event	Any	27 (4.3%)
Cardiac disorders	Any	24 (3.8%)
	Atrioventricular block first degree	4 (0.6%)
	Atrioventricular block second degree	6 (1.0%)
	Bradyarrhythmia	1 (0.2%)
	Bradycardia	10 (1.6%)
	Bundle branch block right	1 (0.2%)
	Sinus bradycardia	2 (0.3%)
Nervous system disorders	Any	2 (0.3%)
	Headache	2 (0.3%)

System Organ Class	Preferred term	N=625 (%)
Vascular disorders	Any	2 (0.3%)
	Hypertension	1 (0.2%)
	Hypotension	1 (0.2%)

Table 2. Summary of patients with adverse events by System Organ Class and Preferred Term, whether or not study drug related (more than one event allowed) (evaluable patients). System Organ Class and Preferred Term according to MedDRA v. 16.1 are displayed.

3.2 Heart rate and its variability

Among evaluable patients, 580 (92.8%) were discharged at the sixth hour after the first dose of fingolimod and only 45 (7.2%, table 3) patients required an extended monitoring. Fingolimod produced a 7.6± 9.2 beats/min average HR reduction from pre-dose value to the sixth hour and the mean relative maximum percentage reduction in HR during the 6-hour monitoring period observed in the study was 17.61± 8.47 % (N=524).

The most frequent reasons for monitoring extension were low heart rate (40%; according to label indications and precautions for use, HR<45b/min or the lowest HR at 6 hours since the first dose) or and cardiac abnormalities observed on ECG (28.9%).

Of the whole population of 625 patients monitored, 542 ECGs were suitable for HRV computation. The remaining 83 traces were not considered sufficiently accurate for the analysis. Of the 542 patients with computed HRV, 39 had prolonged ECG monitoring. Group analyses documented differences between the two groups both in the LF and HF components of HRV (Table 4). In order to estimate the probability of undergoing extended monitoring, univariate logistic regression models were performed on baseline characteristics, setting the median values of the overall populations as cut-offs (Figure 1). Statistically significant ORs were obtained for the following variables:

- Normalized spectral power in the LF band (RR LFnu) <75.2 vs ≥ 75.2 (the median value on the overall population was set as cut-off), OR=2.106 (95%CI: 1.058 – 4.192)
- Normalized spectral power in the HF band (RR HFnu) >=19.4 vs <19.4 (the median value on the overall population was set as cut-off), OR=2.073 (95%CI: 1.041 – 4.126)
- ARR: 0 vs ≥2, OR=3.960 (95%CI: 1.326 – 11.83)

A multivariate regression analysis confirmed, as independent determinants to the need of monitoring extension, only RR HFnu as continuous value [OR (95%CI): 1.023 (1.004 ; 1.043)]

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and the ARR [≥2 vs 0: OR (95%CI): 0.105 (0.021 ; 0.536)]. In line with this, the ARR the year before the study resulted to be statistically significantly different between patients with extended monitoring and patients without extended monitoring (0.9 ± 0.6 vs 1.1 ± 0.7; p=0.0117). Age (p=0.18), disease duration (p= 0.80) and the use of drugs which can alter HR or AV conduction (p=0.13) failed to be significantly associated with the extended monitoring. In particular, 6.1% of subjects under 40 years of age versus 8.8% of subjects over 40 years required an extended monitoring.

Concerning, on the other hand, the potential delay of AV conduction, only 10 (1.6%) patients experienced an AV block (of any degree) during the 6 hours after the first dose of fingolimod. Six out of these 10 patients suffered of a transient 2nd degree AV block but one only was of type 2 in a single brief ECG sequence. Overall, no severe conduction disturbances occurred in any of the treated patients. Interestingly, 1 of the 6 patients who suffered post drug II AV blocks had a HF nu value clearly above the threshold associated with a higher risk of extended monitoring. In the remaining 5 patients, HFnu values were just around threshold or below.

Due to the low number of patients experiencing conduction problems, stratified descriptive analyses were provided whereas logistic regression models were not estimated. All patients experiencing AV blocks were females (p-value of the Fisher exact test 0.0353). Six of them were diagnosed with MS between 3.6 and 9.17 years before the inclusion visit, while four more than 9.17 years before (p-value of the Fisher exact test 0.0280). No other variables presented statistically significant differences, but results have to be interpreted with caution considering the low number of patients with AV blocks.

To evaluate the influence of the individual autonomic profile not only in clinically significant effects but also in the common cardiac effects of fingolimod, a correlation between HRV and the more general HR reduction after fingolimod first dose was performed in the overall population. The correlation analyses between spectral HRV and maximum percentage of HR reduction calculated from the entire post-dosing ECG recordings showed interesting results. Indeed, RR LF nu and RR LF/HF had a weak but significant positive Spearman correlation coefficients (r=0.1394 with p-value=0.0014, and r=0.1654 with p-value=0.0002, respectively) with the relative maximum percentage reduction in HR during the 6-hour monitoring. On the other hand, RR HF nu showed a weak but statistically significant negative Spearman correlation coefficient (r=-0.1835 with p-value<0.0001) with the relative maximum percentage reduction in HR during the 6-hour monitoring. This correlation analyses would suggest a modest inverse correlation between basal vagal tone and actual HR reduction after dosing. However, the very low correlation coefficient observed in this

analysis does not allow, in our view, a solid conclusion about this matter. The overall picture emerging from these analyses shows that an high individual vagal tone, while carrying some implications for the need of extended ECG monitoring due to clinically significant first-dose events, has no influence on the more common HR reduction resolved within the first 6 hours.

It is widely recognized that some cardiac medication and co-morbidities like diabetes mellitus, coronary artery or cerebrovascular diseases have a significant effect on heart rate variability. However, in the present study, the very small number of such conditions suggests a very limited effect, if any, of concomitant therapies on the observed results. Furthermore, high dose intravenous prednisolone might per se cause arrhytmias and influence the autonomic profile of MS patients, but in our sample only 0.6% of subjects were treated with corticosteroids for systemic use in the year before the assessment, ruling out this potential confounding factor from the analysis here presented.

Table 3. Patients not discharged at the end of the first dose monitoring period

		N=45
Reason for non-discharge	Low heart rate ^	18 (40.0%)
	Decreasing heart rate (% respect to baseline)+	4 (8.9%)
	Symptoms associated with bradycardia/bradyarrhythmia	2 (4.4%)
	Cardiac abnormalities observed on ECG*	13 (28.9%)
	Adverse event#	2 (4.4%)
	Other	6 (13.3%)

Table 3. Summary and classification of the events that lead to prolonged patients' monitoring beyond the 6th hour (evaluable patients).

^ HR<45b/min or the lowest HR at 6 hours since the first dose

+ HR reduction respect to baseline, indicated as clinically significant by investigators

* Among the 13 "Cardiac abnormalities observed on ECG", 7 were associated to AV blocks (both 1st and 2nd degree). With regard to the remaining 6 patients, reasons for monitoring extension were reported in the eCRF only for 3 so distributed: "clinical finding unspecified" (2 patients) and "high QTc value" (1 patient). Reasons for extension were not given in the eCRF for 3 patients despite the ECG documented the following events: "increase in RR trend" (1 patient) and "several PVCs, an earlier smaller RR/RRp peak around 3h" (1 patient). Moreover, 1 AV block has been filed in the eCRF as "adverse event".

Adverse event: reason for monitoring extension as indicated in the eCRF.

Table 4. Sympathovagal balance status by monitoring outcome

		N	Mean	SD	25th percentile	Median	75th percentile	Minimum	Maximum	P-value*
Patients who needed to extend the continuous ECG monitoring										
Normalized spectral power in the LF band (RR LFnu)	Yes	39	60.2	26.7	40.7	63.4	80.9	0.0	98.6	0.0424
	No	503	68.7	23.6	56.1	75.6	86.1	0.0	99.5	
	Total	542	68.0	23.9	55.9	75.2	85.8	0.0	99.5	
Normalized spectral power in the HF band (RR HFnu)	Yes	39	33.1	24.4	14.4	24.9	48.0	0.0	94.9	0.0251
	No	503	24.6	20.3	9.1	19.0	34.7	0.0	93.2	
	Total	542	25.2	20.7	9.5	19.5	36.1	0.0	94.9	
Ratio between the absolute spectral power in LF and in HF band (RR LF/HF)	Yes	38	4.3	5.2	0.8	2.7	5.3	0.0	19.0	0.0219
	No	493	6.2	6.1	1.6	3.9	8.5	0.0	19.0	
	Total	531	6.0	6.0	1.5	3.8	8.3	0.0	19.0	

Table 4. Description of the sympathovagal balance status in patients who needed/did not need to extend the continuous ECG monitoring (evaluable patients); *p-value: Wilcoxon Nonparametric Test; SD = standard deviation

Note: In a few cases LFnu or HFnu equal to zero has been observed (LFnu=0, 13 patients, HFnu=0, 11 patients). For patients with HFnu=0 the ratio LF/HF has not been calculated.

4. Discussion

The present study reinforces the evidence about the safety profile of fingolimod with regard to the cardiac side effects. Recently, fingolimod safety has been further validated by a first analysis of an ongoing observational study (START) over almost 4000 patients treated with fingolimod. In the START study bradycardia and advanced AV blocks were seen in as a low 0.8% and 1.6%

respectively of the whole population [14]. Our study adds a novel and significant information to this body of evidence, originating from the careful investigation of the autonomic profile of the MS patients exposed to fingolimod in a large MS population. Correlation between fingolimod and the autonomic system has been previously postulated. A change of sympathetic-vagal balance towards parasympathetic modulation has been shown after fingolimod initiation [6, 7]. The treatment with fingolimod resulted in enhanced vagal activation during the first 6 hours post medication [6, 7] which persisted even after 14 months of treatment [6]. Furthermore, autonomic profile has been suggested to predict fingolimod-induced HR decrease [8, 15], but to date the clinical impact of this correlation has never been investigated. Here we have demonstrated that individual autonomic parameters can predict the need of prolonged observation after fingolimod initiation. In fact, patients with an HF HRV above the median had a higher risk of extended monitoring suggesting a link between individual vagal tone and the risk for fingolimod cardiac effects. An established HF cut-off was not available, therefore the median value was taken into account in order to identify a possible cut-off risk. In particular, from the regression univariate model, patients with HF>=19.4 had a double risk of undergoing extended monitoring versus patients with a lower HF. This was confirmed in the multivariate model, were the HF was considered as continuous variable. Conversely, we failed to demonstrate a positive correlation between HF HRV and post dosing HR reduction. This latter evidence might indicate that vagal activity does not correlate to overall effects of heart frequency, but might specifically identify clinically significant effects leading to monitoring prolongation. In line with this, neither in clinical practice nor in our sample, HR reduction has been considered a cause of monitoring extension. In fact only 9% of patients who needed extended monitoring did so because of HR reduction (see table 3) whereas 40% did so because of low HR. In line with our results, when the cardiac response to fingolimod was investigated as severity of bradycardia, which can be cause of monitoring extension, the extent of parasympathetic response was able to predict cardiac effects [8, 16]. Using complete battery of non-invasive tests for the assessment of cardiovascular reflexes, which included HR variation in response to deep breathing and Valsalva maneuver, the correlation between baseline vagal profile and HR response to fingolimod has been demonstrated [8], but in this case the HR response was investigated as nadir HR and not HR reduction. Furthermore, altered responses to cardiovascular autonomic challenge, prior to fingolimod initiation, were described in all patients who had prolonged HR-slowing beyond the standard first 6 hours after fingolimod administration [16]. Time domain HRV parameters had significant correlation with nadir HR also in the recent study by Li et

al, but in this case the results of decision tree analysis underlined the importance of baseline HR, which was the dominant predicting factor among various demographic, clinical and cardiovascular variables [7]. Cardiac response to fingolimod, even measured by the magnitude of HR decrease after fingolimod initiation, was associated to high parasympathetic cardiac regulation at baseline in one study performed by 24-h ambulatory ECG recording [15].

This is the first study showing that an autonomic nervous system profile characterized by higher vagal modulation, might be related to a higher probability of extending the first dose monitoring, even being confined to a small proportion of patients. In particular, a higher HF power (>19 nu) identified risk for prolonged hospitalization. The absence of an independent relationship between the LF power and prolonged hospitalization is probably explained by the stronger relationship of the related autonomic measure (HF power).

The first dose of fingolimod has been shown to enhance the cardiac parasympathetic activity during the first 6 hours post medication, which might be the underlying autonomic mechanism of reduced heart rate [7]. Nevertheless, this finding is supported by previous evidence as AV conduction is reported to be rather selectively sensitive to modulation of sympathoadrenergic activity, whereas sinus node automaticity is particularly responsive to cholinomimetic influences [17].

In line with this and with our results, Rossi et al showed that the effects on AV conduction, differently from HR reduction, were associated with reduced sympathetic tone and not to parasympathetic indexes, thus indicating the fundamental role of the sympathetic system in limiting the negative dromotropic effects of fingolimod [8]. Of note, the putative synergistic action of the first fingolimod administration and the underlying vagal tone never produced clinically significant cardiac events, as the only 6 cases of 2nd degree AV blocks (1%) were benign and reversible.

On the other hand, the analysis of HR reduction showed a weak inverse correlation with HRV suggesting the possible influence of sympathetic individual tone on fingolimod effect on sinus node. Observing the distribution of HRV parameters shown in figure 1, a large proportion of the studied population had a normalized power in the HF band that was below 50%, thus pointing to a modest prevalence of LF power, reflecting sympathetic predominance. It is worth noting that the autonomic profiles observed in this large study are within the expected findings in a relatively young population [18-21]. The slight sympathetic prevalence (LF nu > 50%) is coherent with the resting but wake condition of all patients, at the time of ECG data collection [21].

Finally, another factor in our study associated to the probability of extension of the monitoring period

beyond 6 hrs after the first dose, was the previous annualized relapse rate. The autonomic balance has been recently shown to be intimately linked with the inflammatory activity of multiple sclerosis, which was featured by an overall hypoactivity of the sympathetic system [22]. The association between autonomic system and disease activity in MS is far to be completely understood and even more complicated is to discuss the implication in fingolimod first dose effects. A disease-specific influence on HRV has been previously shown by the correlation between autonomic balance and disease duration, regardless of the extension of focal demyelination in brain areas controlling these systems, as supposed for years [22]. Here we show the correlation between relapse rate and risk of prolonged hospitalization after fingolimod first dose, independently from disease duration and recent use of corticosteroids. Further investigations are needed to verify the involvement of lesion load and other MRI parameters, related to disease activity, on the association between autonomic balance and fingolimod first dose effects.

5. Clinical Implications

The primary clinical implication of the present study is that fingolimod has minimal cardiac effects and its administration is not associated with threatening cardiac events. The safety data originating from the present study are coherent with previous and current experimental and clinical evidence and indicate that the transient vagomimetic effects of fingolimod are, in most cases, benign and not unusual in otherwise healthy subjects with high vagal tone. Very few cases of significant vagal-like events with fingolimod are reported and were, at most, a symptomatic self-limiting bradycardia (33 beats/min) occurring 9 hours after drug intake [23] and a 7-second sinus arrest in patient treated with risperidone that very likely potentiated the vagomimetic action of fingolimod [24].

The novel aspect emerging from this study is that a background high vagal tone does not amplify the HR rate reduction induced by fingolimod first dose even in those patients with typical signs of vagal dominance. Moreover, our study indicates that HRV, and in particular measures of parasympathetic activity, could be a predictive marker of clinically significant first dose events, which result in prolonged observation after fingolimod initiation, while not being useful to predict the common HR reduction observed during the first 6 hours post dose. It is worth recalling that HRV can be easily and rapidly assessed in clinical setting by short segment ECG recordings.

6. Conclusions

The present study confirms in a large population of patients (625) with MS, carefully ECG monitored over the first 6 hours after its intake, the benign profile of fingolimod, the administration of which has been devoid of significant cardiac consequences. This information adds up to previous information from pivotal and post-marketing studies. The novel information presented here is that the background autonomic profile, and specifically the dominance of cardiac vagal modulation, is not a risky condition for the safe initiation of fingolimod but could help to identify patients whit higher probability to be monitored beyond the first 6 hours. The need of extending cardiovascular monitoring is not a frequent event, and our results could provide useful information for the stratification and individualized monitoring of MS patients prescribed with fingolimod.

7. Acknowledgments
7.1 Ethical standards

7.2 Conflict of interest

8. References

1. Schmouder R, Hariry S, David OJ. (2012) Placebo-controlled study of the effects of fingolimod on cardiac rate and rhythm and pulmonary function in healthy volunteers. Eur J Clin Pharmacol; 68:355–362.

2. Guo J, MacDonell KL, Giles WR. (1999) Effects of sphingosine 1-phosphate on pacemaker activity in rabbit sino-atrial node cells. Pflugers Arch; 438(5):642-648.

3. Schmouder R, Serra D, Wang Y, Kovarik JM, DiMarco J, Hunt TL, Bastien MC. (2006) FTY720: placebo-controlled study of the effect on cardiac rate and rhythm in healthy subjects. J Clin Pharmacol.; 46:895–904.

4. Montano N, Porta A, Cogliati C, Costantino G, Tobaldini E, Casali KR, Iellamo F. (2009) Heart rate variability explored in the frequency domain: a tool to investigate the link between heart and behavior. Neurosci Biobehav Rev.; 33:71–80.

5. Hilz MJ, Wang R, de Rojas Leal C, Liu M, Canavese F, Roy S, Hösl KM, Winder K, Lee DH, Linker RA. (2017) Fingolimod initiation in multiple sclerosis patients is associated with potential beneficial cardiovascular autonomic effects. Ther Adv Neurol Disord. 10(4):191-209

6. Akbulak RÖ, Rosenkranz SC, Schaeffer BN, Pinnschmidt HO, Willems S, Heesen C, Hoffmann BA. (2018) Acute and long-term effects of fingolimod on heart rhythm and heart rate variability in patients with multiple sclerosis. Mult Scler Relat Disord.; 19:44-49.

7. Badilini F, Pagani M, Porta A. (2005) HeartScope: a software tool addressing autonomic nervous system regulation. Computers in Cardiology.; 32:259–262.

8. Li K, Konofalska U, Akgün K, Reimann M, Rüdiger H, Haase R, Ziemssen T. (2017) Modulation of cardiac autonomic function by fingolimod initiation and predictors for fingolimod induced bradycardia in patients with multiple sclerosis. Frontiers in Neuroscience; 11: 1-9.

9. Rossi S., Rocchi C, Studer V, Motta C, Lauretti B, Germani G, Macchiarulo G, Marfia GA and Centonze D. (2015) The autonomic balance predicts cardiac responses after the first dose

of fingolimod. Mult Scler J; 21 (2): 206-216.

10. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation. 1996; 93:1043–1065

11. Malliani A, Pagani M, Lombardi F, Cerutti S. (1991) Cardiovascular neural regulation explored in the frequency domain. Circulation.; 84:482–492

12. Piha SJ. (1991) Cardiovascular autonomic reflex tests: Normal responses and age-related reference values. Clin Physiol; 11: 277–290.

13. Mathias CJ and Bannister R. (1999) Investigation of autonomic disorders. In: Mathias CJ and Bannister R (eds) Autonomic failure. A textbook of clinical disorders of autonomic nervous system. 4th ed. Oxford: Oxford University Press, pp.169–195.

14. Limmroth V, Ziemssen T, Lang M, Richter S, Wagner B, Haas J, et al. (2017) Electrocardiographic assessments and cardiac events after fingolimod first dose – a comprehensive monitoring study. BMC Neurol.; 17:11.

15. Simula S, Laitinenb TP, Laitinenb TM, Hartikainenc P, Hartikainend JEK. (2016) Heart rate variability predicts the magnitude of heart rate decrease afterfingolimod initiation. Mult Scler Relat Disord; 10: 86–89.

16. Hilz MJ, Wang L, de Rojas Leal C, Liu M, Canavese F, Roy S, Hosl KM, Winder K, Lee DH, Linker RA. (2017) Fingolimod initiation in multiple sclerosis patients is associated with potential beneficial cardiovascular autonomic effects. Ther Adv Neurol Disord., Vol. 10(4) 191– 209.

17. Carruthers SG, McCall B, Cordell BA, Wu R. (1987) Relationships between heart rate and PR interval during physiological and pharmacological interventions. Br J Clin Pharmacol; 23:259–265.)17.

18. Kuo TB, Lin T, Yang CC, Li CL, Chen CF, Chou P. (1999) Effect of aging on gender differences in neural control of heart rate. Am J Physiol.; 277: H2233–2239

19. Wang SY, Zhang LF, Wang XB, Cheng JH. (2000) Age dependency and correlation of heart rate variability, blood pressure variability and baroreflex sensitivity. J Gravit Physiol.; 7:P145–146.

20. Antelmi I, de Paula RS, Shinzato AR, Peres CA, Mansur AJ, Grupi CJ. (2004) Influence of age, gender, body mass index, and functional capacity on heart rate variability in a cohort of subjects without heart disease. Am J Cardiol.; 93:381–385

21. Montano N, Ruscone TG, Porta A, Lombardi F, Pagani M, Malliani A. (1994) Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. Circulation. 1994; 90:1826–1831.

22. Studer V, Rocchi C, Motta C, Lauretti B, Perugini J, Brambilla L, Pareja-Gutierrez L, Camera G, Barbieri FR, Marfia GA, Centonze D, Rossi S. (2017) Heart rate variability is differentially altered in multiple sclerosis: implications for acute, worsening and progressive disability. Multiple Sclerosis Journal _Experimental, Translational and Clinical.; 5;3(2):2055217317701317. doi: 10.1177/2055217317701317.

23. Faber H, Fischer HJ, and Weber F. (2013) Prolonged and symptomatic bradycardiafollowing a single dose of fingolimod. Mult Scler; 19 (1): 126-128 .

24. Espinosa PS and Berger JS. (2011) Delayed fingolimod-associated asystole. Mult Scler; 17: 1387-1389.

Figure Legends

Figure 1. Determinant factors of monitoring extension.

The figure shows a univariate logistic regression model on demographic, clinical and autonomic variables. Normalized spectral power in the HF band (marking vagal modulation), Normalized spectral power in the LF band (marking sympathetic modulation) and relapse rate were significantly correlated with the probability of undergoing extended monitoring.

- The safety of fingolimod first dose is confirmed in a large piopulation of patients
- The need of extending the first dose monitoring is a rare event
- individual autonomic profile predicts the need of prolonged observation.

Determinant factors of monitoring extension
Odds Ratio and 95% CL

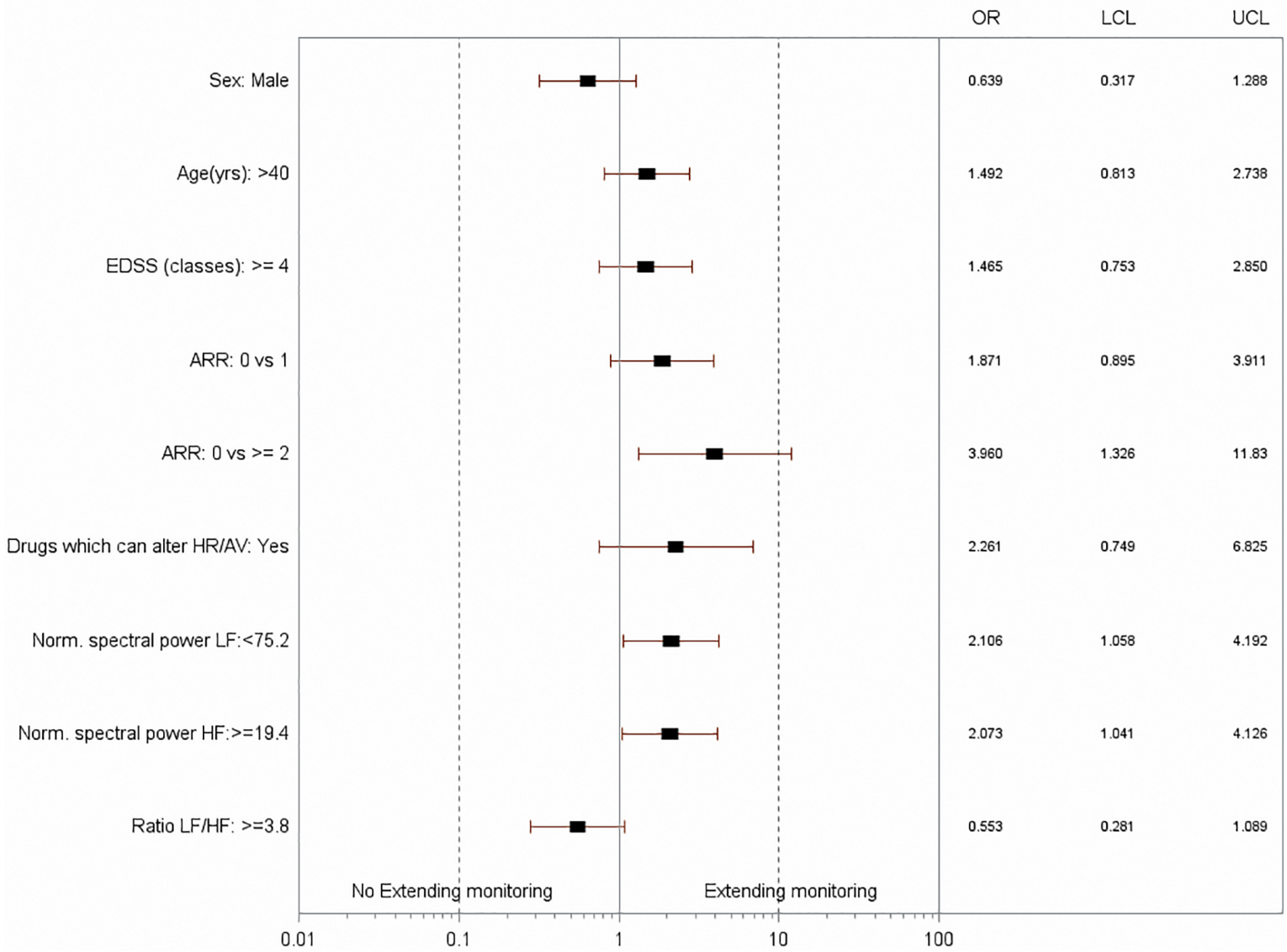|	OR	LCL	UCL
Sex: Male	0.639	0.317	1.288
Age(yrs): >40	1.492	0.813	2.738
EDSS (classes): >= 4	1.465	0.753	2.850
ARR: 0 vs 1	1.871	0.895	3.911
ARR: 0 vs >= 2	3.960	1.326	11.83
Drugs which can alter HR/AV: Yes	2.261	0.749	6.825
Norm. spectral power LF:<75.2	2.106	1.058	4.192
Norm. spectral power HF:>=19.4	2.073	1.041	4.126
Ratio LF/HF: >=3.8	0.553	0.281	1.089

No Extending monitoring   Extending monitoring

0.01   0.1   1   10   100

Figure 1