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Correlation between IL-31 and sCD40L plasma levels in Fingolimod-treated patients with Relapsing-Remitting Multiple Sclerosis (RRMS)

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ABSTRACT

Introduction: Multiple Sclerosis (MS) is a chronic, autoimmune, demyelinating disease of the central nervous system (CNS). Currently, several protocols are described for the different phases of MS. In this longitudinal study, we aim to quantify the concentration of plasma cytokines of MS patients treated with Fingolimod alone or after Glatiramer Acetate (GA) or Interferon-beta (IFN-β), in order to compeer both treatments and describes if it is possible to use them as biomarkers.

Objective: Compare the two different types of drug treatment and describes possible immune biomarkers in RRMS patients treated with Fingolimod alone or after GA or IFN-β.

Materials and methods: This is a controlled, non-randomized clinical trial. Plasma concentrations of IL-31, sCD40L and nine others cytokines were evaluated in two groups of patients with a one-year follow-up. Group 1 ($n = 12$): RRMS patients treated with GA or IFN-β for at least six months before the study who changed therapy to Fingolimod after six months, and Group 2 ($n = 12$): naïve RRMS patients who started treatment with Fingolimod. We used ANOVA two-way to analyze the cytokines and Spearman coefficient to evaluate the correlation.

Results: Although Group 2 started with a greater number of relapses per disease duration, Fingolimod treatment was effective in decreasing this parameter, as well as EDSS over 12 months. However, the treatment with GA or IFN-β on Group 1 showed a tendency to increase the number of relapses after 6 months of follow-up, which decrease when the therapy was changed to Fingolimod. After the evaluation of 11 cytokines in one year, we found that IL-31 and sCD40L were the biomarkers that demonstrated a more difference when compared to the classical ones, following the clinical pattern over the treatment period.

Conclusions: Our study describes the existence of two promising plasmatic biomarkers (IL-31 and sCD40L), which reduced plasmatic levels in RRMS patients followed the treatment time of Fingolimod, despite that more studies are needed to prove their efficiency.

1. Introduction

Multiple Sclerosis (MS) is the most prevalent chronic inflammatory autoimmune disease of the central nervous system (CNS) and is linked to a variety of environmental factors (Ascherio and Munger, 2016; Fragoso, 2014), including smoking (Handel et al., 2011), lack of vitamin D (Simpson Jr et al., 2018), obesity (Kvistad et al., 2015) and previous contact with Epstein Barr virus (Maghzi et al., 2011). MS is neurodegenerative disease and the immunopathogenesis is related to the activation of different T-lymphocyte clones against the myelin sheath

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through molecular mimicry or by activation through scattering of epitopes at the periphery (McMahon et al., 2005). On the othe hand, B-cell activation in the form of oligoclonal bands (OCB) production is the most consistent immunologic finding in patients with MS (Bar-Or et al., 2010; Maghzi et al., 2011).

The production of cytokines such as TNF-α, IL-12, IL-6, IL-23 and IL-1β are very important in polarization of T helper cells, which can be Th1 or Th17, involving in the pathogenesis of MS (Rothhammer et al., 2011). It is important to mention that patients with MS have abnormal immunoglobulin values, mainly due to increased synthesis and oligoclonal bands in cerebrospinal fluid. Another noteworthy point is the B cells of these patients, who are more leaning to the production of proinflammatory cytokines such as IL-6, TNF-α and GM-CSF, are deficient in the regulation of IL-10, suggesting greater activation of Th1 and Th17 cells, besides myeloid cell activation through GM-CSF (Bar-Or et al., 2010).

Currently, there is no cure for MS, the treatments are focused on reducing the risk of relapsing and disability progression (Rae-Grant et al., 2018). Over the years new disease-modifying therapies (DMT) in the immune reconstruction therapy emerged and the clinicians have several MS treatment options available, with different mechanisms of action, risk profile and monitoring requirements. But all of them has their risk associated and clinicians need to evaluated the benefit-risk to ongoing MS and those relates to the different treatment options (Rae-Grant et al., 2018).

The BRACE treatment (Betaferon®, Rebif®, Avonex®, Copaxone®), is widely approved as a DMT for MS, and comprise Glatiramer acetate (GA) and beta-interferon (IFN). They have less risk and are effective for many patients, however almost one-quarter of patients with RRMS receiving BRACE continue to present relapse activity, causing the switch of treatment (Alsop et al., 2017).

Fingolimod (Gilenya®) was the first approved oral drug for the treatment of relapsing-remitting forms of MS (Thomas et al., 2017). Acting in a new approach, fingolimod is a high affinity agonist of sphingosine 1-phosphate (S1Ps) (Eken et al., 2017), allowing the internalization of type 1 S1P receptors (Noguchi and Chun, 2011), thus inhibiting the lymphocyte traffic to the systemic circulation (Lee et al., 2010; Henault et al., 2013), and consequently reducing their infiltration in the SNC (Cohen and Chun, 2011).

There are some differences in the guideline uses of Fingolimod, while in USA, Australia and Switzerland it can be used as first-line therapy without any restrictions. In European Union, Canada and Brazil, Fingolimod is used as second-line therapy in patients that fail to respond to at least one previous treatment or in patients with rapidly onset MS (Rae-Grant et al., 2018; Alsop et al., 2017).

However, the timing of the treatment transition still be uncertain. Thinking on this, the objective of this study is to relate the cytokines concentration present in the blood of MS patients (before and after fingolimod treatment) with clinical evidence, suggesting new possible biomarkers for the exchange of therapy.

2. Methods

2.1. Outline

Controlled, non-randomized, clinical trial. We evaluated plasmatic concentrations of several cytokines, including IL-31 and sCD40L, in three different moments over a 1-year period, in 12 RRMS patients being treated with GA or IFN-β at least six months before study and changed treatment to Fingolimod in the sixth month of study (Group 1) and 12 RRMS patients who were previously naïve and started treatment with Fingolimod (Group 2). **Inclusion criteria:** 18 to 60-year old patients diagnosed with RRMS according to modified 2010 McDonald's criteria (Polman et al., 2011); naïve or being treated with IFN-β or GA for at least six months prior to the inclusion in the study; scored 0–5.5 in Kurtzke Expanded Disability Scale (EDSS) and with last relapse determined more than 3 months prior to study beginning. **Exclusion criteria:** patients with progressive MS forms, using corticoids less than 3 months prior to study beginning, patients over 55 years, with other neurological diseases, diabetes, neoplasms, reumathological diseases, abusive usage of alcohol or illegal drugs were excluded. **Clinical data collection:** Each patient had three visits, where they went through a 3.0-Tesla Magnetic Resonance (MRI scan) of the Brain and peripheral blood sampling, with a 6-month period between visits. Blood samples were collected upon clinical evaluation in the morning period, from 8 am to 12 pm, in order to decrease deviations linked to changes in the circadian cycle of sampled cytokines. A patient from Group 1 withdrew from the study after the second visit and he was then removed. Samples were immediately stored in a − 80 °C ultra-freezer for further processing. The plasma levels of sCD40L, IL-1β, IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-31, IL-33, TNF-α and IFN-γ were evaluated by Bio-Rad®'s Bio-plex Pro Human Th17 CytokineAssay kit (Cat. #171AA001M). **Statistical Analysis:** The two-way analysis for repeated measures (*ANOVA two-way*) was used to compare the variable of difference between the first and last moment of all biomarkers. The test of repeated measurements was used along with Bonferroni's *post hoc* in order to enable the harmonization between the variables for further correlation study through Spearman coefficient. A $p < 0.05$ significance level was applied. **Ethical Aspects:** This clinical essay followed the guidelines established by the 2013 Helsinki Declaration. This study meets the requirements stated by National Health Surveillance Agency (ANVISA) – RDC no. 36/2012 for the execution of Clinical Researches. All participants have signed the Informed Consent Form.

3. Results

To start our experiments, we separate our patients in two different groups: patients being treated with GA or IFN-β and changed treatment to Fingolimod in the first 6 months (Group 1) and patients who were naïve and started immediately treatment with Fingolimod (Group 2). Both groups were observed for one year at three different time points and their cytokines and clinical evidences were analyzed. In Table 1, it is possible to assess the clinical variables of both groups. Regarding disease

Table 1
Association of study variables between both groups.

Characteristic	Group 1	Group 2	*p*-value
Gender$_1$	12	12	0.680
Male	6 (50.0)	4 (33.3)	
Female	6 (50.0)	8 (66.7)	
Previous time of disease$_2$ (months) ($n_1 = 12$; $n_2 = 12$)	55.50 (13.0–86.25)	13.0 (7.25–27.75)	0.060
Age (years)$_3$ ($n_1 = 12$; $n_2 = 12$)	28.29 (±7.80)	30.46 (±8.15)	0.512
EDSS 1stvisit$_3$ ($n_1 = 12$; $n_2 = 12$)	2.25 (±1.47)	2.88 (±1.69)	0.345
EDSS 2ndvisit$_3$ ($n_1 = 12$; $n_2 = 12$)	2.13 (±1.32)	2.58 (±1.40)	0.417
EDSS 3rd visit$_3$ ($n_1 = 11$; $n_2 = 12$)	1.96 (±1.31)	2.04 (±0.94)	0.856
No. relapses/disease duration$_1$ 1st visit$_4$	0.667	1.500	<0.001*
No. relapses/disease duration$_1$ 2nd visit$_4$	0.833	0.500	0.19
No. relapses/disease duration$_1$ 3rd visit$_4$	0.182	0.167	1.00

Average (±standard deviation); Median (1st– 3rd quartile); Frequency (%); $_1$Fisher's exact test; $_2$Mann Whitney's test; $_3$Student's *t*-test; $_4$Z test for comparison of proportions.

activity, one may conclude that only in the first visit a difference between groups in quantity of relapses per year was noticeable, as it was significantly larger in Group 2 ($p < 0.001$). There was also no significant difference between groups when comparing EDSS independently on the visit (1st $p = 0.345$, 2nd $p = 0.417$ and 3rd $p = 0.856$).

When analyzing each group separately, comparing the 12-month period, a significant decrease in number of relapses per year is noticeable in Group 2 ($p < 0.001$). Similarly, an impressive decline in EDSS for Group 2 is noticeable perceptible after 12 months when compared to the base period ($p = 0.050$).

The results of all measured biomarkers (in pg/ml) are described on Table 2. No statistical difference was identified when comparing both groups on the three visits. However, when evaluating groups separately, we noticed Group 1 showed reduced levels of IL-31 and sCD40L after 12 months of treatment ($p < 0.001$). It is even more interesting to observe the treatment change in Group 1, from GA and IFN-β to Fingolimod led to a decrease in IL-31 plasmatic levels after 6 months of treatment (p = 0.05). In Group 2, in turn, IL-31 and sCD40L reported a significant decline after 12 months of Fingolimod treatment, $p = 0.002$ and $p < 0.001$, respectively. The decrease of these two biomarkers was sharper in the last 6 months of Fingolimod therapy: $p = 0.048$ and $p = 0.017$, respectively (Fig. 1).

Other cytokines have reported no statistical difference, however some of them shows a correlation with IL-31 and sCD40L demonstrating a similar pattern. In Group 1, IL-31 correlated positively with IL-33 ($r = 0.762$, $p = 0.028$) and sCD40L with IL-1b ($r = 0.800$, $p = 0.003$) and IL-17A ($r = 0.782$, p = 0.04), as described on Table 3. In Group 2, sCD40L correlated with TNF-α ($r = 0.882$, $p < 0.001$), IL-17A ($r = 0.443$, $p < 0.001$), IL-17F ($r = 0.881$, $p = 0.004$), IL21 ($r = 0.688$, $p = 0.019$) and IFN-γ ($r = 0.605$, $p = 0.032$) and IL-31 correlated negatively with the amount of weighted lesions in sequence in T2/FLAIR ($r = -0.591$, $p = 0.043$), as seen on Table 4.

These results indicate that the IL-31 and sCD40L could be correlated with the common proinflammatory cytokines (IL-1β, IL-17A and IFN-γ) and Fingolimod efficacy, acting as a possible biomarker for treatment response. It is worth mentioning that more studies are needed to prove this hypothesis.

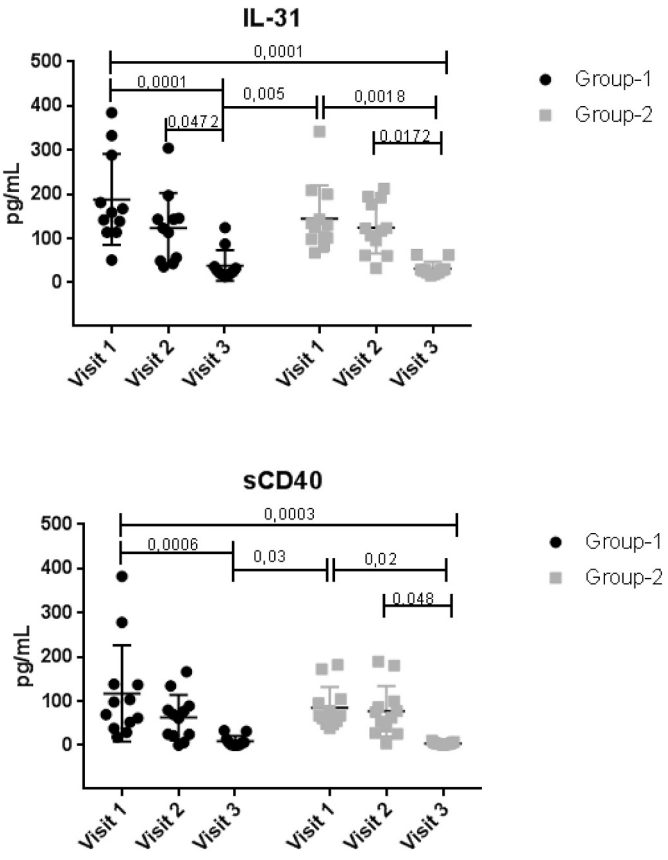

Fig. 1. Fingolimod treatment decreases the concentration of inflammatory cytokines in RRMS patients.
After the analysis period, the plasma samples taken from patients in Group 1 (RRMS previously treated with GA or IFN-β) and Group 2 (RRMS previously untreated) at baseline (Visit 1), in 6 months (Visit 2) and in 12 months (Visit 3) follow-up. A) IL-31 plasma concentration. B) sCD40L plasma concentration. Analysis performed through Anova two-way test. N of 12 patients.

4. Discussion

In this article we compare two different types of drug treatment and describes possible immune biomarkers in RRMS patients treated with Fingolimod alone or after GA or IFN-β. First of all, we compare the rate of relapses per year the rate of relapses per year in both groups to investigate if Fingolimod as a first-choice medication (Group 2) would represent an improvement in clinical evolution when compared to those who started the treatment with other medications as GA or IFNβ and switched to fingolimod in first 6 months (Group 1).

Although there is no difference between those groups in the end of one-year period, is worth reinforcing that patients on Group 2 has no treatment before the beginning of this study, and so started the treatment with a significantly larger relapse rate ($p < 0.001$), representing a steeper decrease in these numbers (Group 1, difference of 0.485; Group 2, difference of 1.333). Such findings suggest a better therapeutic response in Group 2 after one-year treatment with Fingolimod. Also related to the rate of relapses per year, Group 2 patients reported further EDSS decline over the treatment period ($p = 0.05$), due to length of use longer than 6 months of a therapy known to be more efficient than IFN-β, as seen in TRANSFORMS study (Cohen et al., 2010).

When considering the cytokine dosages, the plasmatic levels identified in sCD40L and IL-31 doesn't shows differences between the groups

Table 2
Values of biomarkers in both groups on the 3 visits in pg/ml.

Marker	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Pg/ml	Visit 1	Visit 1	Visit 2	Visit 2	Visit 3	Visit 3
sCD40L	117.14 ± 30.16	84.70 ± 13.03	62.88 ± 14.14	76.85 ± 15.89	9.10 ± 3.48	4.29 ± 0.83
IL-31	188.47 ± 29.55	144.84 ± 20.77	123.22 ± 22.95	124.62 ± 16.19	38.98 ± 9.95	31.45 ± 4.23
IL-22	2.54 ± 0.75	1.40 ± 0.19	1.09 ± 0.30	1.91 ± 0.18	1.54 ± 0.70	0.42 ± 0.10
IL-1β	0.32 ± 0.87	0.40 ± 0.14	0.17 ± 0.52	0.30 ± 0.08	0.09 ± 0.03	0.18 ± 0.06
IL-6	2.49 ± 0.62	2.31 ± 0.60	2.56 ± 0.65	1.63 ± 0.38	2.12 ± 0.61	1.63 ± 0.27
IL-17A	0.52 ± 0.12	0.68 ± 0.25	0.48 ± 0.19	0.50 ± 0.16	0.27 ± 0.10	0.56 ± 0.19
IL-17F	4.79 ± 0.97	5.78 ± 1.65	3.79 ± 1.26	4.06 ± 1.07	4.56 ± 0.95	4.61 ± 1.35
IL-21	19.08 ± 5.86	23.09 ± 9.98	16.85 ± 11.41	13.37 ± 4.85	7.69 ± 4.04	9.38 ± 3.54
IL-33	5.81 ± 1.51	5.19 ± 1.58	6.43 ± 1.90	3.81 ± 0.93	6.34 ± 1.51	2.66 ± 0.75
TNF-α	5.65 ± 0.71	3.99 ± 0.40	5.37 ± 0.94	3.52 ± 0.27	3.62 ± 0.67	2.63 ± 0.26
IFN – γ	3.33 ± 0.51	2.98 ± 0.22	1.84 ± 0.90	2.47 ± 0.93	1.07 ± 0.51	1.39 ± 0.75

Table 3
Correlation between IL-31 and sCD40L biomarkers in Group 1.

Characteristic in Group 1	Spearman'srho	sCD40L	IL-31
sCD40L	Correlation coefficient	–	0.445
	p value	–	0.170
TNF-alfa	Correlation coefficient	0.509	−0.100
	p value	0.110	0.770
Number of weighted lesions in T2/ FLAIR	Correlation coefficient	−0.151	0.073
	p value	0.658	0.831
Volume of weighted lesions in T2/ FLAIR	Correlation coefficient	0.096	0.106
	p value	0.778	0.757
IL-31	Correlation coefficient	0.445	1.000
	p value	0.170	–
No. relapses/disease duration	Correlation coefficient	0.509	0.155
	p value	0.110	0.650
IL-22	Correlation coefficient	0.500	0.500
	p value	0.253	0.253
IL-1 β	Correlation coefficient	0.800*	0.055
	p value	0.003	0.873
IL-6	Correlation coefficient	−0.600	−0.409
	p value	0.051	0.212
IL-17A	Correlation coefficient	0.782*	0.173
	p value	0.004	0.612
Il-17F	Correlation coefficient	0.500	0.464
	p value	0.253	0.294
IL-21	Correlation coefficient	0.612	−0.067
	p value	0.060	0.855
IL-33	Correlation coefficient	0.429	0.762*
	p value	0.289	0.028
IFN – γ	Correlation coefficient	0.600	0.491
	p value	0.055	0.125
EDSS Difference	Correlation coefficient	0.005	−0.333
	p value	0.988	0.317

* $p = 0.05$.

Table 4
Correlation between IL-31 and sCD40L biomarkers in Group 2.

Characteristic in Group 2	Spearman's rho	sCD40L	IL-31
sCD40L	Correlation coefficient	1.000	0.191
	p value	–	0.574
TNF-alfa	Correlation coefficient	0.882**	−0.098
	p value	0.000	0.762
Number of weighted lesions in T2/ FLAIR	Correlation coefficient	0.481	−0.591*
	p value	0.135	. 043
Volume of weighted lesions in T2/ FLAIR	Correlation coefficient	0.468	0.304
	p value	0.146	0.338
IL-31	Correlation coefficient	−0.191	1.000
	p value	0.574	–
No. relapses/disease duration	Correlation coefficient	0.318	−0.427
	p value	. 340	. 167
IL-22	Correlation coefficient	0.300	0.322
	p value	0.433	0.364
IL-1β	Correlation coefficient	0.527	−0.545
	p value	0.096	0.067
IL-6	Correlation coefficient	.-264	−0.161
	p value	0.433	0.618
IL-17A	Correlation coefficient	0.433**	−0.500
	p value	0.000	0.667
IL-17F	Correlation coefficient	0.881*	0.083
	p value	0.004	0.831
IL-21	Correlation coefficient	0.688*	−0.137
	p value	0.019	0.672
IL-33	Correlation coefficient	0.430	−0.118
	p value	0.214	−0.729
IFN – γ	Correlation coefficient	0.645*	−0.413
	p value	0.032	−0.183
EDSS Difference	Correlation coefficient	0.274	−0.575
	p value	0.415	0.055

* $p = 0.05$.
** $p = 0.001$.

in the first dosage. Even though there was no statistical difference between both groups in third visit ether, the levels of those cytokines are lower when compared to the first one in Group 1 ($p < 0{,}0006$; p ⟨0,0001) and Group 2 ($p < 0{,}02$; $p < 0{,}0018$). Those results are probably due to Fingolimod's therapeutic effect once it induces sCD40L decreasing (Kornbluth et al., 1998) through reducing T lymphocyte numbers (Henault et al., 2013) and consequently the ability to activate mast cell, B-lymphocytes, as well as other antigen-presenting cells (Dong et al., 2014; Skaper et al., 2017), such as macrophages and dendritic cells (Taylor et al., 2012).

CD40 is a membrane protein from the receptor's superfamily of tumor necrosis factor (TNF-α) (Karnell et al., 2019), expressed by immune and non-immune cells, and its CD40 ligand (CD40L) is expressed in a transitory form, particularly on the membrane surface of activated T-helper CD4+ lymphocytes. CD40L is crucial in modulating several autoimmune processes (Kim et al., 2011a), at which T and B-lymphocytes co-stimulation play a role in MS pathogenesis (Laman et al., 1998; Masuda et al., 2017). The soluble form of the ligand (sCD40L) has similar properties to the non-soluble one (Karnell et al., 2019) and its circulating level reflects the CD40-CD40L proinflammatory system activation (Vakkalanka et al., 1999; Smagina et al., 2015).

The IL-31 is the main proinflammatory cytokine involved in cutaneous allergic reactions, stimulating the release of granules in mast cells (MCs) (Saleem et al., 2017). MCs can infiltrate the CNS and interact with astrocytes, microglia and endothelium through the release of proinflammatory cytokines (Dong et al., 2014; Skaper et al., 2017). Those cells mediate the inflammation and demyelination, introducing myelin antigens to T-lymphocytes, modulating of the blood-brain barrier (BBB) permeability and increasing the influx of cells and inflammatory cytokines to CNS (Dong et al., 2014; de et al., 2019). The correlation between IL-31 and CNS mast cell activation are described in several neuroinflammatory processes (Skaper et al., 2017; Che et al., 2018) including MS (Forsythe, 2019).

When analyzing both groups, there has been a significant correlation between sCD40L and IL-31 along the 12 months of treatment ($r = 0.834$, $p < 0.001$). These data are compatible with the findings of Guerrero-Garcia (Guerrero-garcía et al., 2017), the first description of the reduction of sCD40L and Il-31 in patients of MS. At the end of the same year, Barcutean & Romaniuc (Bărcuţean et al., 2018) corroborated these results, indicating a proinflammatory co-modulation of IL-31 / sCD40L axis in MS' physiopathology, possibly through mast cell activation (Dong et al., 2014).

Guerrero-Garcia (Guerrero-garcía et al., 2017) and Barcutean & Romaniuc (Bărcuţean et al., 2018) suggest IL31/sCD40L axis inhibition is possibly a therapeutic target in RRMS and its secondary progressive

form (SPMS) (Bărcuţean et al., 2018). In addition, it is important to mention that serum levels of IL-31 and sCD40L, as well as, mRNA levels for CD40, tend to decrease with the time of disease progression (Kim et al., 2011b).

We suggest that, besides the peripheral blockade to lymphocytic migration, the plasmatic level reduction in this axis might also be related to another immunological action caused by Fingolimod (Yan et al., 2005). This DMT has been recently linked to an anti-mastocytic action in murine models of rhinitis (Kleinjan et al., 2013), dermatitis (Tsuji et al., 2012), asthma (Lai et al., 2011) and intestinal allergy (Blázquez et al., 2010). This possibly occurs through induction of apoptosis (Kurashima et al., 2007), by activating a way that does not depend on S1P receptor, inhibiting A2 phospholipase (Payne et al., 2007). So far, studies in human beings evaluating this possible anti-mastocytic response through Fingolimod are unknown.

In MS, IL-1β and IL-17 are proinflammatory interleukins linked to Th1 and Th17-lymphocytes, respectively (Prajeeth et al., 2017). Barcutean & Romaniuc (Bărcuţean et al., 2018) demonstrated a negative correlation between IL-1β and IL-17 serum levels and the disease duration time, pointing out to the crucial role of these cytokines in MS' initial immune cascade. Our study correlated these cytokines reduction with sCD40L, suggesting an inhibiting action common to both sCD40L and these cytokines, likely due to circulating Th1 and Th17-lymphocyte reduction by Fingolimod (Sato et al., 2014), with consequent decrease of lymphocytic infiltration in CNS (Cohen and Chun, 2011). Also secreted by mast cells, IL-1β is a cytokine which has been related to the physiopathology of several neuroinflammatory processes (Bonnekoh et al., 2018). Its reduced level linked to sCD40L may imply a decrease in mast cell activity by Fingolimod.

TNF-α and IFN-γ levels have correlated with sCD40L reduction levels. TNF-α and IFN-γ are among the main proinflammatory cytokines secreted by Th1 lymphocyte and are jointly related to MS physiopathology (Sato et al., 2018). Fingolimod is active on IFN-γ through a Th1 decrease in response (La Mantia et al., 2016), justifying plasmatic reduction.

IL-17F is one of the most important cytokines released by Th17 lymphocytes (Qu et al., 2013), most likely because it modulates the expression of cytokines that cause pro-inflammatory effects when linked to Th1 response, such as TNF-α (Korn et al., 2009). High serum levels of IL-17F have been associated with a suboptimal response to IFN-β in RRMS patients (Sato et al., 2014). Our findings have shown a correlation between sCD40L and IL-17F, possibly due to the fact that CD40-CD40L complex activation influences lymphocyte differentiation in Th17 (Iezzi et al., 2009). Fingolimod reduces the serum level of proinflammatory cytokines secreted by Th17 lymphocyte, possibly through peripheral sequestration of these cells (Henault et al., 2013; Cohen and Chun, 2011), thus justifying IL-17A and IL-17F reduced plasmatic levels throughout the study. Therefore, Th17 response reduction by Fingolimod might, as well, be also a consequence of an anti-sCD40L activity.

Our study found a correlation between sCD40L and IL-21 plasmatic levels in Group 2. Th17 lymphocytes release IL-21, which is another proinflammatory interleukin that activates follicular T-helper lymphocytes and natural killer (NK) cells (Ghalamfarsa et al., 2016), stimulates B-lymphocyte differentiation (Yoshizaki et al., 2012) and suppresses T-regulator lymphocyte differentiation. IL-21 has been seen as a driving force for autoimmune diseases, including MS (Tzartos et al., 2011). Our study has found a correlation between the plasmatic levels of such biomarkers, suggesting IL-21 reduction levels might likewise be linked to Th17 response reduction by Fingolimod.

Recent studies have been correlating IL-31 with IL-33, suggesting that the presence of one interleukin might stimulate the other, thus amplifying neuroinflammation through co-stimulation, known as IL-31/IL-33 axis (Di Salvo et al., 2018). IL-33 is an interleukin that belongs to IL-1 family, which also includes IL-1α and β, who play an important part in typical inflammation, occurred in allergic diseases mediated by Th2 lymphocytes (Stott et al., 2013). In MS, it is postulated that the IL-31/IL-33 axis might activate and induce disease progression mediated by mast cell activity (Di Salvo et al., 2018). Therefore, as previously suggested, plasmatic reduction in this axis is possibly justifiable by Fingolimod's anti-mastocytic action.

In this study, IL-31 levels correlated inversely with the total amount of weighted lesions in sequence in T2/FLAIR, possibly due to maintenance of activity in the MRI scan, mainly at the expense of accumulation of new T2/FLAIR weighted lesions, due to residual activity in the MRI scan. Such findings support similar results found in TRANSFORMS extension studies, showing disease activity in scanning despite the therapy with fingolimod or interferon beta-1a (Cohen et al., 2016).

Barcutean & Romaniuc (Bărcuţean et al., 2018) suggest IL-31/sCD40L axis inhibition is possibly a therapeutic target in RRMS and its secondary progressive form (SPMS). Through the significant correlations between sCD40L and illness duration and between IL-31 and EMRR patients' age, the authors suggest there is a possible age-dependent co-modulation in these patients. Differently from our study, the authors followed a larger number of patients through a longer period, possibly justifying the findings of the correlation between sCD40L and duration of disease.

In a prospective study evaluating 29 RRMS patients using Natalizumab (NTZ) for 8 months, Balasa & Simu (Balasa et al., 2017) have shown IL-31 serum levels were reduced in relation to the controls. These findings correlated inversely with disease duration and time of treatment and positively with the number of relapses before treatment. sCD40L correlated inversely with the number of relapses before treatment and positively with age, suggesting these biomarkers might be linked to a therapeutic response to NTZ. In an unprecedented way, our study additionally suggests IL-31 and sCD40L levels reduction might be related to Fingolimod's immunological activity in humans and to a therapeutic response as a consequence.

However, our study has a few limitations, such as a restricted sample of patients, a short 12-month follow up, and the absence of a control group formed by healthy patients, besides the fact that we did not correlate the plasmatic level to cerebrospinal fluid. Despite those limitations, as seen in other studies recently published (Guerrero-garcía et al., 2017; Bărcuţean et al., 2018; Balasa et al., 2017) we call attention to IL-31 and sCD40L in MS pathogenesis and suggest these cytokines could be biomarkers for Fingolimod action during MS.

5. Conclusions

Our study suggests the existence of two promising plasmatic biomarkers, IL-31 and sCD40L, which reduced their plasmatic levels in RRMS patients treated with Fingolimod. Further studies are necessary in order to enlighten the correlation of such biomarkers in MS physiopathology and in therapeutic response to Fingolimod.

Declaration of Competing Interest

This academic study is financially supported by Novartis. The authors do not receive any reimbursement or financial benefits and declare that they have no competing interests. Novartis played no role in the study design, methods, data management or analysis, or in the decision to publish.

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