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ORIGINAL COMMUNICATION

Treatment with alemtuzumab or rituximab after fingolimod withdrawal in relapsing–remitting multiple sclerosis is effective and safe

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Abstract

Background It has been described that treating relapsing–remitting multiple sclerosis (RRMS) patients with alemtuzumab following fingolimod could be less effective due to the different dynamics of lymphocyte repopulation. Effectiveness and safety of alemtuzumab compared to rituximab after fingolimod withdrawal were analyzed.

Patients and methods A follow-up of a cohort of RRMS patients treated with alemtuzumab or rituximab after fingolimod withdrawal was accomplished. Effectiveness, measured by the percentage of patients with no evidence of disease activity (NEDA), and the presence of side effects (SE) were registered.

Results Fifty-five patients, 28 with alemtuzumab and 27 with rituximab, were analyzed. No differences in the washout period or in the baseline lymphocytes counts were observed. After a mean follow-up period of 28.8 months, the annualized relapsing rate was significantly reduced in the alemtuzumab group from 1.29 to 0.004 ($p < 0.001$) and in the rituximab group from 1.24 to 0.02 ($p < 0.001$), without differences. A significant reduction of the median EDSS from 2.8 to 2.0 in the alemtuzumab group and from 3.5 to 2.5 ($p < 0.01$) in the rituximab group was observed, without differences. Eighty-two per cent ($n = 28$) of patients in alemtuzumab group and 69.2% ($n = 26$) in rituximab group achieved NEDA criteria, without differences ($p = 0.3$). Symptoms related to the infusion were the most frequent SE in both groups. No serious SE were registered.

Conclusion Treating RRMS patients with alemtuzumab or rituximab after fingolimod withdrawal is effective and safe, without significant differences between both groups in our series.

Keywords Relapsing–remitting multiple sclerosis · Treatment · Alemtuzumab · Rituximab · Fingolimod withdrawal · Lymphocyte repopulation

Introduction

Multiple sclerosis (MS) MS is a chronic, inflammatory, and demyelinating disorder mediated by self-reactive T and B cell attacks within the central nervous system (CNS) [1, 2]. Nowadays, highly effective modifying treatments (DMT), traditionally known as second-line therapies, are available for aggressive naïve relapsing–remitting multiple sclerosis (RRMS) patients and for RRMS patients that fail to first-line therapies [3–5]. These therapies have different mechanisms of action such as interference with immune cell trafficking or lymphocyte function and lymphocyte depletion [6–9]. Although their efficacy and safety, in naïve patients or after first-line therapies, have been widely studied, there are currently no controlled studies/trials of switching between second-line therapies due to lack of efficacy or safety issues. Therefore, the place and timing of different second-line treatments used sequentially are nowadays guided by observations of clinical practice [10, 11]. However, this issue must be continuously assessed as new therapies are being available and as it is important to know the immediate and long-term consequences of sequential therapy in terms effectiveness and safety in the short and long term.

Fingolimod is a DMT widely used as a second-line treatment in RRMS in naïve patients with aggressive disease or after first-line drug failure [12–15]. Fingolimod produces a maintained lymphopenia as a result of prolonged lymph

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node sequestration of lymphocytes that may persist even 2 months after its withdrawal [16].

Alemtuzumab is a humanized anti-CD52 monoclonal antibody (MoAb) approved for the treatment of active RRMS, in both naïve patients or after failure of first- and second-line treatments [17–20]. As the main pharmacologic action, alemtuzumab targets the cell-surface antigen CD52 that is expressed on more than 95% T and B cells. This interaction induces lysis of peripheral lymphocytes, leading to their rapid and prolonged depletion from the circulation [21].

Rituximab is an anti-CD20 MoAb capable of destroying B cells, via complement and antibody-dependent cellular cytotoxicity, largely administrated out of label in MS patients who experienced disease activity on standard therapies [22–24].

It has been postulated that effectiveness of alemtuzumab following fingolimod may be compromised. Alemtuzumab would fail to suppress inflammatory activity as a likely result of the different dynamics of lymphocyte repopulation after fingolimod withdrawal [25, 26].

The objective of this study is to analyze the effectiveness and safety of alemtuzumab after fingolimod therapy. As a comparison group, we have selected, a cohort of patients treated with rituximab, following fingolimod therapy.

Patients and methods

To be eligible, all RRMS patients from two University Hospital MS Units from the Hospital Universitari i Politècnic La Fe (HUPLF) and the Hospital Clínic Universitari (HCU), two tertiary hospitals in Valencia (Spain) that fulfilled the following criteria were included: (1) MS diagnosis as per the McDonald 2010 diagnostic criteria [27]; (2) received treatment with alemtuzumab or rituximab after fingolimod withdrawal; (3) a minimum follow-up period from fingolimod discontinuation of 6 months.

In all cases treated with rituximab, approval from both the Spanish Medicines Agency of the Spanish Ministry of Health (Agencia Española de Medicamentos y Productos Sanitarios–AEMPS) and the local Ethical Committee from each hospital were obtained, as stated in the Spanish Royal Decree for compassionate use of medicines (RD 1015/2009, June 19th).

Study design and procedures

An observational, retrospective study of the clinical and radiological characteristics of RRMS patients treated with alemtuzumab and rituximab following fingolimod withdrawal was carried out, analyzing data included in the GITEM Registry of patients (*Grup d'Investigació i Tractament de l'Esclerosi Múltiple*), approved by the Health Department of the Valencian Autonomous Region (Health Regional Authority–Conselleria de Sanitat-), and the data collected by both MS Units during clinical visits in the follow-up period.

The criteria to initiate alemtuzumab or rituximab in RRMS patients were the presence of a suboptimal response to fingolimod (see definition of treatment failure below) or suffering side effects with previously aggressive disease. Rituximab was selected as an active comparator because, as alemtuzumab, it is a high-efficacy monoclonal antibody with lymphocyte-depleting activity. At first, in all RRMS patients with suboptimal response to fingolimod, rituximab was administrated out of label, because there were no other available options at that moment (in most of these patients natalizumab had been previously withdrawn due to progressive multifocal leukoencephalopathy-PML-risk). After availability of alemtuzumab in November of 2014, most patients were switched from fingolimod to this second-line treatment.

All patients were assessed in routine clinical practice. At the time the decision to start the new treatment was confirmed (considered as baseline demography data age at the first attack of MS and gender) and retrospective clinical data (presence of IgG or IgM-oligolonal bands-OCB-, previous DMT used, annualized relapse rate—ARR-, score of the Expanded Disability Status Scale (EDSS) the year before and at baseline, and baseline radiological activity) were collected. At baseline, a complete blood analysis including platelets, red and white cell blood recount, hepatic, renal, and thyroid function, viral serologic assessment (hepatitis B and C virus, human immunodeficiency virus), and screening for latent tuberculosis infection, were performed in all patients.

Washout period between fingolimod discontinuation and new treatment start was not previously defined in the study protocol, but in our clinical practice it was approximately between 4 and 6 weeks. Alemtuzumab was given as two annual courses on five consecutive days at baseline and on three consecutive days 12 months later, as it is recommended in the package leaflet of the medicinal product. In this group we used a modified pre-medication scheme to prevent infusion-associated reactions, consisted of 1 g/day of IV methylprednisolone throughout all 5 days of alemtuzumab treatment, associated to 1 g of paracetamol and 5 mg of dexchlorpheniramine. During the 1st month under alemtuzumab, prophylactic acyclovir (200 mg/12 h) according to prevent viral infections was administrated.

In the rituximab group, at first, a dose of 1000 mg intravenous rituximab was administered twice, on day 1 and day 15. A more detailed explanation of the protocol of administration is found elsewhere. Briefly, for maintenance, an isolated dose of 1000 mg was administered when the percentage of total CD19 + cells was 2% or more [24]. Intravenous pre-medication to prevent allergic reaction to

the infusion consisted of 100 mg of prednisolone, 1 gram of paracetamol, and 5 mg of dexchlorpheniramine.

Both treatments were administered at the outpatient facilities by trained nurses. The attending neurologist collected all infusion-related adverse effects. After alemtuzumab administration, scheduled clinical visits were planned every month for the first 3 months and every 3 months later. A blood and urine test was done every month, with thyroid function and lymphocyte subpopulations counts every 3 months. After rituximab, scheduled clinical visits and complete blood test, including lymphocyte subpopulations counts, were planned every 3 months. During these visits the neurologist registered the presence of new symptoms, EDSS, and potential side effects. A MRI scan was performed 6 and 12 months from the first administration of alemtuzumab, and, after that, with annual periodicity. In the group of rituximab, an annual MRI scan was performed in most patients (except for patients whom follow-up period was less than 1 year).

Effectiveness of alemtuzumab and rituximab was measured by the percentage of patients who met the definition of NEDA (no evidence of disease activity).

Definitions

A *relapse* was defined by the presence of new suggesting neurological symptoms, maintained for more than 24 h, in the absence of intercurrent processes and accompanied by objective changes in neurological examination. A disabling relapse was defined as one producing a worsening of at least 1 point in the EDSS and leaves a residual disability score of at least 2.0.

Confirmed disability progression and confirmed improvement of disability (CDP, CID) were defined by a decrease or increase, respectively, in one point in EDSS (if EDSS was less than 6) or in 0.5 point (if EDSS was 6 or more) persisting after 6 months.

Clinical activity was defined as the presence of relapses and/or sustained progression of disability and *radiological activity* was defined as the presence of new T2 and/or gadolinium-enhancing lesions (GEL) in a MRI scan.

Secondary-progressive MS was diagnosed in those patients with a minimum EDSS score of 4.0 with a pyramidal functional system score of 2, when an increase of 1 point in the EDSS was detected out of relapses and this increase was confirmed in a subsequent 6-month separated clinical visit.

Treatment failure was defined as the presence of one of the following: (1) two relapses in 1 year; (2) one no-disabling relapse and presence of ≥ 1 GEL in a MRI acquired at least 3 months after the beginning of the clinical relapse; (3) one disabling relapse.

No evidence of disease activity (*NEDA*) was considered if absence of clinical and radiological activity was demonstrated.

Statistic analysis

SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) 21.0.v and GraphPad Prism v5.01 were used. Kaplan–Meier survival analysis for the median time to a relapse and to increase of disability was run.

Results

Baseline characteristics

At April 2018, 55 patients with a minimal follow-up of 6 months after fingolimod withdrawal were considered, 35 from the HUPLF and 20 from the HCU. Twenty-eight patients received alemtuzumab and 27 patients rituximab. Forty-two were women (76.4%) and the median age at first attack of MS was 24.5-year-old (standard deviation-SD 7.7). Most patients had IgG-OCB in the CSF (42/50, 84%) and more than a half had IgM-OCB (24/42, 57.1%). Thirty-nine patients (72.2%) had one or more GEL in the baseline MRI (Table 1).

The median of previous received treatments was different between both groups, being greater for rituximab, with a median of three previous treatments (interquartile range-IQR 1–3), compared to alemtuzumab, with a median of 2 (IQR 1–3; $p=0.006$) (Table 1).

The mean disease duration from the first relapse to the new treatment was also different between groups, being longer for rituximab, 11.4 years (SD: 6.2) with respect to alemtuzumab, 7.8 years (SD 5.5; $p=0.04$) (Table 1). The mean EDSS a year before new treatment was 2.3 (SD 1.1), and at baseline, it had increased to 2.9 (SD 1.2). The mean ARR 1 year before starting new treatment was 1.2 (SD 0.9). There were no differences in EDSS or ARR between both groups (Table 1).

Despite treatment with fingolimod, 50 patients (90.9%) had an active disease during the year previous to rituximab or alemtuzumab, fulfilling criteria of treatment failure. In the remaining five patients, fingolimod was withdrawn due to side effects and alemtuzumab or rituximab was indicated for previously aggressive disease.

No differences in the washout period were observed between groups, 42 days for alemtuzumab (IQR 21.3–59.5), and 34 days for rituximab (IQR 17.3–61.3; $p=0.49$); or in the number or total lymphocytes before starting the new treatment, 1310 u/μL for alemtuzumab (IQR 815–1670), or 1100 u/μL for rituximab (IQR 665–1580; $p=0.44$) (Table 1).

Table 1 Baseline clinical and demographic characteristics of the total of the series and of alemtuzumab and rituximab separately

	Alemtuzumab ($n=28$)	Rituximab ($n=27$)	Total series ($n=55$)	p^a
Age at first relapse	26.1 (7.3)	22.7 (7.9)	24.5 (7.7)	0.18
Age at ALT/RTX onset	34.1 (7.2)	34.6 (8.3)	34.4 (7.7)	0.66
Sex (% females)	75.0	77.8	76.4	0.8
OCGB (*n*, % positive)	20/25 80.0	22/25 88.0	42/50 84	0.45
OCMB (*n*, % positive)	11/19 57.9	13/23 56.5	24/42 57.1	0.93
ARR the year before	1.25 (0.9)	1.15(0.8)	1.2 (0.9)	0.54
EDSS the year before	2.1 (0.9)	2.6 (1.2)	2.3 (1.1)	0.14
EDSS at baseline	2.8 (1.0)	3.1 (1.3)	2.9 (1.2)	0.23
Patients with GEL at BL	77.8	66.7	72.2	0.38
Mean time to ALT or RTX (y.)	7.8 (5.5)	11.4(6.2)	9.7 (6.1)	0.04
Previous treatment before FGM	2 (1–3)	3 (1–4)	2 (2–4)	0.006
Washout period (d., IQR)	42 (21.3–59.5)	34 (17.3–61.3)	40 (20–60.3)	0.45
Baseline lymphocytes recount (IQR)	1310 (815–1670)	1100 (665–1580)	1256 (766.3–1597.5)	0.44
Median time after FGM withdrawal IQR)	21.7 (13.8–36.2)	32.5 (11.4–50)	22.3 (12.8–41.9)	0.33
Median time under ALT or RTX (IQR)	18.1 (11.8–31.4)	32.0 (10.6–48.6)	20.9 (11.8–38.7)	0.27

Comparisons were made between different groups, indicating *p* value and highlighting where there are significant differences ($p<0.05$)

OCGB oligoclonal B bands, *OCMB* oligoclonal M bands, *ARR* annualized relapsing rate, *GEL* gadolinium-enhanced lesion, *BL* baseline, *ALT* alemtuzumab, *RTX* rituximab, *FGM* fingolimod, *y* year, *d* days, *IQR* interquartile range

Alemtuzumab and rituximab effectiveness

After fingolimod withdrawal, all patients were followed up at least for 6 months; 39 (70.9%) were followed for more than 1 year and 27 (49.1%) for more than 2 years. The median follow-up time was 22.3 months (IQR 12.8–41.9), without differences between groups; and the median follow-up time under new treatment was 20.9 months (IQR 11.8–38.7), also without differences between groups (Table 1). In alemtuzumab group, a control MRI scan after treatment start was performed in all patients, in 20 patients (71.4%) after 1 year and in 8 patients (28.6%) after 6 months. In rituximab group, a MRI scan after treatment start was done in all patients but 1 (96.3%), in 23 patients (88,5%) after 1 year and in three patients after 6 months (11.5%).

The ARR was significantly reduced in the alemtuzumab group from 1.29 to 0.004 ($p<0.001$) and in the rituximab group from 1.24 to 0.02 ($p<0.001$); no statistical differences were found between groups (Fig. 1).

After 1 year of follow-up, a significant reduction of the median EDSS (interquartile range) from 2.8 (2–3) to 2.0 (1.5–2.5) ($p=0.03$) in the alemtuzumab group and from 3.5 (2–4) to 2.5 (2–4) ($p<0.01$) in the rituximab group was observed. These reductions remained stable after the second year, without statistical differences between both groups (Fig. 2).

Eighty-two per cent ($n=28$) of patients in the alemtuzumab group and 69.2% ($n=26$) in the rituximab group achieved NEDA criteria, without differences ($p=0.3$).

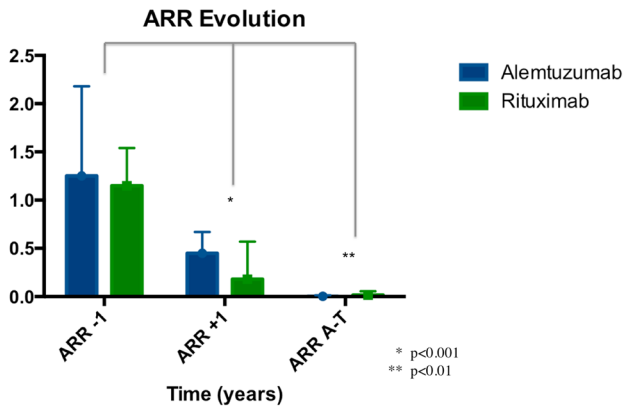
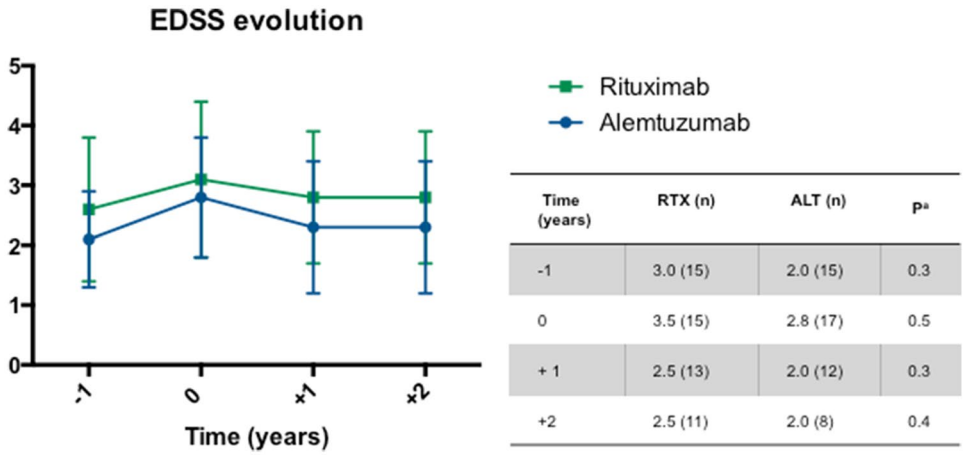


Fig. 1 Evolution of annualized relapsing rate, 1 year before and after treatment (1 year and at last visit). Data corresponding to alemtuzumab and rituximab separately are shown. A significant reduction of ARR was observed for both groups. No differences between both groups were observed in any period of time (ARR −1, ARR+1, ARR A-T). *ARR −1* annualized relapsing rate 1 year before treatment, ARR+1 annualized relapsing rate 1 year after treatment, ARR A-T annualized relapsing rate after treatment (at last visit)

There were no differences in the percent of patients free from relapses, radiological activity or CDP (Table 2).

Baseline lymphocyte counts and washout period were not different between patients who had inflammatory activity after alemtuzumab or rituximab and those who did not (Table 3).



Time (years)	RTX (n)	ALT (n)	P[a]
-1	3.0 (15)	2.0 (15)	0.3
0	3.5 (15)	2.8 (17)	0.5
+ 1	2.5 (13)	2.0 (12)	0.3
+2	2.5 (11)	2.0 (8)	0.4

Fig. 2 Evolution of disability measured by EDSS, before and after the 1st and 2nd year under both treatments, alemtuzumab and rituximab. A significant increase in EDSS had been observed the year before starting new treatment. For both groups, alemtuzumab and rituximab, a significant decrease in EDSS was observed after the first year of treatment, which was maintained during the second year. *RTX* rituximab, *ALT* alemtuzumab

Table 2 Disability, inflammatory activity (clinical and radiological) and NEDA criteria after treatment, of the total of the series and of alemtuzumab and rituximab separately

	Alemtuzumab ($n=28$)	Rituximab ($n=27$)	Total series ($n=55$)	p^a
Confirmed improvement of disability (n, %)	10 (35.7)	7 (25.9)	17 (30.9)	0.43
Free from confirmed worsening of disability (%)	27 (96.4%)	26 (96.4)	53 (96.4)	0.98
Free from relapses (%)	26 (92.9)	22 (81.5)	48 (87.3)	0.21
Free from radiological activity (%)	24 (85.7)	21 (80.8)	45 (83.3)	0.63
NEDA criteria (%)	23 (82.1)	19 (70.4)	42 (76.4)	0.30

Comparisons were made between different groups, indicating p value. Significative differences were not observed between both groups

NEDA no evidence of disease activity

Table 3 Contingency table comparing the presence or absence of inflammatory activity respect to basal lymphocyte counts and washout period, in the total of the series and in alemtuzumab and rituximab groups separately

	Inflammatory activity YES ($n=11$)	Inflammatory activity NO ($n=44$)	Total series ($n=55$)	p^a
Baseline lymphocyte counts	1056 (680–1310)	1310 (782–1630)	1256 (766.3–1597.5)	0.3
Washout period (days)	38 (15–70)	38 (20.5–58.5)	40 (20.5–60.5)	0.9

Comparisons were made between different groups, indicating p value. Significative differences were not observed between groups

Tolerability and safety

Despite pre-medication with steroids and antihistamine drugs, symptoms related to the infusion were the most frequent side effects (SE) in both groups, in 16 patients (57.1%) for alemtuzumab and in 7 (29.2%) for rituximab. Headache and cutaneous rash were the most frequent symptoms related to the infusion in both groups. Fever and headache were the most common side effects on 1st day and skin rash was the most common on 5th day of therapy. In a minority of patients, asymptomatic bradycardia and hypotension were present for all 5 days of therapy. In most cases the symptoms were mild and did not require the interruption of the treatment during the infusion.

One patient who received rituximab suffered symptoms compatible with serum sickness, successfully managed with symptomatic treatment, but rituximab was no longer administered.

During the follow-up, 14 (51.9%) patients for alemtuzumab and 5 (18.5%) for rituximab had common infections (respiratory and urinary) with good recovery with standard treatment. None of the patients developed severe or opportunistic infections. As expected, the most frequent infection in alemtuzumab group was herpes simplex.

Thyroid dysfunction was observed in 4 patients (14.3%) who had received alemtuzumab. One patient suffered an atrial fibrillation secondary to hyperthyroidism that was resolved with symptomatic treatment and the rest of patients

were asymptomatic. In 1 patient with alemtuzumab, a severe ($< 500/mm^3$) but transient neutropenia was observed more than 1 year after treatment infusion, maybe in relation to an autoimmune mechanism, but antibodies against neutrophils were not tested. Monthly analytics were performed, and neutropenia was resolved spontaneously after 4 months. One patient, with rituximab and concomitant oral contraceptives, suffered a deep venous thrombosis with secondary mild pulmonary embolism. Complete recovery with anticoagulant therapy was observed and oral contraceptives were withdrawn. One patient was diagnosed of breast neoplasm 3 months after rituximab infusion. She started chemotherapy and rituximab was no longer administered. In other patient rituximab was withdrawn due to persistent and uncontrollable pruritus. Main side effects are summarized in Table 4.

Withdrawal of alemtuzumab and rituximab treatment

Alemtuzumab was withdrawn in one patient due to CDP 2 years after last infusion of treatment, in the absence of inflammatory activity. Diagnosis of secondary-progressive multiple sclerosis (SPMS) was performed and compassionate treatment with rituximab was initiated.

Rituximab was withdrawn in six patients. In two patients, treatment was withdrawn for suboptimal response, having inflammatory activity (relapses and new GEL), and a bone marrow transplantation was performed in both cases. In two other patients, rituximab was withdrawn due to adverse effects; one for serum sickness and another for persistent pruritus. The first patient returned to natalizumab (treatment that was initiated previously to fingolimod) assuming PML risk and, in the other one, a de-escalation therapy to teriflunomide was done. In one patient treatment was discontinued because of safety concern after diagnosis of breast cancer. In the last case, rituximab was switched to ixekizumab, a monoclonal antibody that targets the interleukin 17A, with the purpose to control a concomitant aggressive psoriasis that was active despite rituximab. Rituximab was no longer administered in this patient, as concomitant treatment with ixekizumab has not been studied in terms of safety.

Discussion

In our real-world data of this cohort of MS patients with an aggressive form of the disease, followed for a median time of 20.9 months, alemtuzumab was safe and useful for controlling the inflammatory activity and the progression of short-term disability in patients with RRMS following fingolimod. Alemtuzumab helped to achieve NEDA status after fingolimod treatment, without differences compared to rituximab, another treatment that has been shown to be effective in aggressive forms of MS.

Baseline characteristics were similar for both groups, except to the median of previous received treatments and the mean disease duration from the first relapse to the new treatment, being both greater for rituximab. These two differences can be explained because rituximab is an off-label treatment, so standard treatments might have been prioritized, thus delaying the start of the drug. Nevertheless, despite these differences, the response to treatment following fingolimod was similar between both groups.

The clinical phase III studies CARE-MS I and II revealed high efficacy of alemtuzumab compared to interferon-β-1a treatment with ARR reduction of 55 or 49%, respectively [17, 18, 28]. Results were recently corroborated by long-term data generated from various extension studies [20, 29]. So, nowadays, alemtuzumab is mainly used for the treatment of RRMS with high disease activity.

Table 4 Main side effects, in the total of the series and in alemtuzumab and rituximab groups separately

Side effect	Alemtuzumab ($n=28$)	Rituximab ($n=27$)	Total series ($n=55$)	p^a
Related to the infusion	16 (57.1%)	7 (29.2%)	23 (41.8%)	0.04
Serum sickness	0	1(3.7%)	1 (1.8%)	
Common infections	14 (51.9%)	5 (18.5%)	19 (34.6%)	0.001
Opportunistic infections	0	0	0	
Autoimmunity	5 (17.9%)	0	5 (9.1%)	0.05
Thyroid disfunction	4 (14.3%)			
Cytopenias	1 (3.6%)			
Renal disfunction	0			
Trombotic event	0	1 (3.7%)	1 (1.8%)	
Cancer	0	1 (3.7%)	1 (1.8%)	
Others	0	1	1 (1.8%)	
Persistent pruritus	0	1		

Comparisons were made between different groups, indicating p value. Symptoms related to the infusion, common infections and presence of autoimmunity were significantly more frequent in alemtuzumab group with respect to rituximab group

Insufficient control of disease activity may require switching from fingolimod to alemtuzumab, but studies that analyze the effectiveness and safety of this switch provide controversial information [25, 26]. An unexpected high disease activity after fingolimod withdrawal without starting any treatment immediately after its discontinuation has been described, similar to natalizumab [30–32]. It has been hypothesized that starting alemtuzumab after fingolimod could further potentiate the rebound effect by depleting circulating regulatory cells [16, 33–35]. It has also been described that treating RRMS patients with alemtuzumab following fingolimod could be less effective due to the different dynamics of lymphocyte repopulation [25]. The occurrence of an unexpected inflammatory disease activity after alemtuzumab induction following fingolimod has been reported, suggesting that prolonged lymph node sequestration of autoreactive lymphocytes following fingolimod withdrawal prevents these cells from the usual biological effect of alemtuzumab, limiting its efficacy [25]. In this study, 9 out of 36 analyzed patients were found to have persistent clinical and/or MRI disease activity within one year after the first course of alemtuzumab, with a mean washout period of 6 weeks (range 2.5–10), similar to our series. Pre-treatment lymphocyte counts were found to have a great variability: 4/9 patients had normal lymphocyte count (> 1000/μL) and 4/9 patients had a moderate lymphopenia (> 400/μL). In our series, only 1 patient had a significant lymphopenia (240/μL) with a washout period of 5 weeks. Lymphocyte counts would be expected to normalize 2–4 weeks after fingolimod withdrawal [16]; however, our series as described in others [25, 26], shows the variability in the rate of recovery of lymphocyte counts among patients. As a matter of fact, in our series and that of Willis et al., patients with a more pronounced lymphopenia previous to new treatment did not necessarily have had a shorter washout period [25].

In contrast, another study describes high efficacy of alemtuzumab in a cohort of RRMS with high inflammatory activity despite being treated with fingolimod. In this series, similar circumstances of lymphocyte counts dynamics are described, but a longer mean washout period, approximately 19 weeks [26], was applied. These authors suggested that a longer washout interval, without signs of persistent or severe lymphopenia, allowed alemtuzumab to be more effective in this subgroup of patients.

In our series, the median washout period was 6 weeks, similar to the Willis et al. study [25] and median lymphocyte counts rised above the lower normal limit (1256 lymphocytes, in contrast to 940 lymphocytes of the other study). In our cohort, no relation of lymphocyte counts with disease activity under alemtuzumab treatment was found. Furthermore, it must be highlighted that the longer the washout period after fingolimod withdrawal the higher the risk of new disease inflammatory activity. Also, we want to emphasize that a rebound syndrome has been described after fingolimod withdrawal [33–36]. There is also a case reported of multiple sclerosis reactivation after switching from fingolimod to rituximab [36]. In this case the washout period was about 6 weeks and the reactivation was early, only 19 days after rituximab infusion; after that the patient was stable for the rest of follow-up time, 8 months. Authors emphasized that repeated dosing 2 or 4 weeks after first infusion of rituximab, as we have done, could control this phenomenon, depleting late B cells egressing from secondary lymphoid organs. In our study, fingolimod was withdrawn due to suboptimal response in most cases, so waiting beyond 6 weeks to alemtuzumab when the disease is not controlled may suppose a high risk of new relapses or MRI activity. No differences in the washout period or in the lymphocyte counts before starting the new treatment (alemtuzumab or rituximab) were observed in our cohort. Most of our patients had normal baseline lymphocyte counts after this washout period, while most of reported cases of activity after switch to alemtuzumab were related to low baseline lymphocyte counts [25, 26]. In our series, five patients (45.5% of all patients who had inflammatory activity) presented new relapses or GEL during the first 6 months after alemtuzumab or rituximab, and, in this subgroup, the lymphocyte counts at baseline and after washout period were similar to the rest of the cohort. Nonetheless, disease activity of alemtuzumab after fingolimod withdrawal related to low lymphocyte count has been described specially in the first months of treatment [25, 26].

With all, disease activity with alemtuzumab after fingolimod withdrawal might be more related to low lymphocyte baseline counts rather than washout periods and consequently confirmation of lymphocyte repopulation may be advisable before starting alemtuzumab. Studying factors that may influence the different times to lymphocyte repopulation after fingolimod withdrawal, such as immunosenescence [37] or previous DMT among others, maybe could be helpful to identify these patients. In our opinion, we must take into account both factors, washout period and lymphocyte counts at baseline, before starting alemtuzumab after fingolimod, and try to balance them.

Like alemtuzumab, rituximab treatment after fingolimod withdrawal was also highly effective to treat a RRMS subgroup with high disease activity, without differences to reach NEDA criteria.

Alemtuzumab following fingolimod was also safe in our cohort, without any unexpected or atypical side effect under appropriate pre-medication and treatment monitoring. As described in the CARE-MS I and II studies, symptoms related to the infusion were the most frequent SE [17, 18, 28, 38]. We used a modified pre-medication scheme to prevent infusion-associated reactions consisting of 1 g/day of IV methylprednisolone throughout all

5 days of alemtuzumab treatment, associated to 100 mg of paracetamol and 5 mg of dexclorpheniramine [39]. As expected, rates for severe infusion-associated reactions (< 2%) and severe infections (< 3%) were not different in our cohort [38].

Thyroid dysfunction was observed in 4 patients (14.3%) who had received alemtuzumab. This proportion is similar than that reported in literature, and most patients were asymptomatic and only abnormal thyroid function parameters in blood tests were found [40]. No other autoimmune diseases were documented in alemtuzumab or rituximab groups.

Compared to the control cohort of RRMS patients with similar baseline characteristics treated with rituximab, no difference in the washout period, baseline lymphocyte counts, and treatment effectiveness measured by reaching NEDA criteria was found. SE observed under rituximab were also consistent with what has been described in the literature, being the most frequent SE symptoms related to the infusion [22, 24].

We are conscious that our study has several limitations, mainly the retrospective design, the limited patient numbers, and the short follow-up time after the first course of alemtuzumab. Although it would advisable to confirm these results in a prospective, randomized, multicentre study due to these unavoidable potential biases, this could be difficult and costly to implement. On the other hand, real-life data studies can add novel information about safety, lymphocyte repopulation dynamics and can help in the design of future studies comparing efficacy of high-efficacy drugs, so collecting evidence from observational studies is paramount.

Due to the aforementioned limitations, it is not possible to exclude whether any baseline demographic variables (like previous treatment received or different follow-up evolution time before fingolimod) could contribute to explain the response of different treatments after fingolimod. Also, it remains unknown whether shorter washout periods or lower baseline lymphocyte counts than described in our study may affect the efficacy and safety of alemtuzumab. Despite our limitations, in the absence of interventional, randomized studies, we conclude that treating RRMS patients with alemtuzumab or rituximab after fingolimod withdrawal is effective and safe.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical standard All patients included in the study acceded to donate and signed a specific informed consent and all research was conducted following legal and ethical requirements at the Research Institute of the Hospital La Fe and was approved by its Institutional Review Board.

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