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ORIGINAL ARTICLE

Post-natalizumab clinical and radiological findings in a cohort of multiple sclerosis patients: 12-month follow-up

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Abstract There is an urgent need to identify the best strategies to prevent the loss of natalizumab (N) beneficial effects after its suspension. The objective is to evaluate the clinical and radiological disease activity and to test the efficacy of immunomodulatory/immunosuppressive drugs (IT) after N suspension. Clinical and radiological data from 54 patients 2 years before treatment (pre-N), during treatment (on-N) and after interruption, during 1-year follow-up (post-N) were retrospectively collected. Annualized relapse rate (ARR), expanded disability status scale (EDSS), presence of new T2 lesions and Gd+ (gadolinium enhancing) T1 lesions were evaluated. Pre-N ARR at 1 year was 1.74 while post-N ARR was 0.94 ($p = 0.0053$). We observed that post-N disease activity never raised over pre-N levels, neither post-N ARR nor post-N EDSS. In patients retreated with N after suspension, post-N ARR was significantly lower than pre-N ARR ($p = 0.017$), but not in patients treated with other IT or in patients not treated with any disease modifying drugs (DMD). The mean time of freedom from new T2 lesions and new Gd+ lesions was lower in post-N period compared to on-N (T2 lesions $p = 0.0000$, Gd+ lesions $p = 0.0000$). In conclusion, a "rebound" pattern was not identified in our cohort, though the disease activity rapidly returned after N, regardless of the treatment used.

Keywords Natalizumab · Drug suspension · Multiple sclerosis · Rebound · Drug-holidays · IRIS

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Introduction

Natalizumab (N) is a very efficient monoclonal antibody used in relapsing remitting multiple sclerosis (RRMS) [1, 2]. However, despite its high efficacy, N is considered a second line treatment in RRMS, because of the risk of progressive multifocal leukoencephalopathy (PML), a potentially fatal infection [3]. Nowadays the risk of PML is believed to depend on immunosuppressive treatments carried out at an earlier stage, on the exposure of the JC-virus and, most of all, on the length of N treatment, due to a reduction in CNS immunological defenses [4]. Consequently, N cannot be considered a long-life treatment and the true open question lies on the effects of its discontinuation.

At first, with regards to PML-risk, drug-holidays were supposed to temporarily re-establish the physiological immune system functions. Patients put on drug-holidays unfortunately experienced severe clinical and radiological relapses and consequently some authors started to believe that the important increase of the disease activity after treatment interruption was due to a "rebound" phenomenon [5, 6].

According to several studies, the MRI findings after the N suspension might be very similar to IRIS, the immune reconstitution inflammatory syndrome that occurs after antiretroviral therapy in HIV seropositive patients [7]. Accordingly the ending of N activity could lead to a rapid

reconstitution of the lymphocytes trafficking and to an important CNS inflammation, leading sometimes to an IRIS-like pattern [8]. Nowadays, a single definition of "rebound" does not exist yet, however, it is generally considered as a change in the disease course with worsening of the disease activity beyond the pre-treatment levels.

We collected the clinical and radiological data of 54 patients, all previously treated with N, on a follow-up time of 1 year after interruption. The aim of this study was to evaluate the consequences of N suspension, comparing the clinical and radiological data with those collected before and during N treatment, and to investigate the effect of different therapeutic strategies after N suspension.

Patients and methods

Patients

The administration of N was carried out at the Multiple Sclerosis Center of Cagliari. In Italy at the time of the study, according to the Regulatory Authorities Recommendations modified by the Italian Pharmaceutical Agency–AIFA,N was indicated for the following RRMS patients: *CRITERION-A*, patients with at least 2 relapses in the previous year while on immunomodulant treatment (at least 12 months), or 1 relapse with uncomplete remission and residual disability not <2 on expanded disability status scale (EDSS), and who had at least 9 T2-hyperintense lesions in cranial MRI or at least 1 Gd+ (gadolinium enhancing)-lesion; *CRITERION-B*, patients with 2 or more relapses in 1 year with uncompleted remission and residual disability not <2 on EDSS, and with 1 or more new Gd+ enhancing lesions on brain MRI or a significant increase in T2 lesion load as compared to a recent MRI in the last year [9].

The reasons for the interruption were different: drug holyday, pregnancy desire, fear of secondary effects. Those patients who developed anti-N antibodies were excluded from the analysis. An indirect comparison of the clinical and radiological data was made considering the 3 different observational periods: (1) before treatment (pre-N); (2) during treatment (on-N); and (3) after the treatment suspension (post-N).

Clinical data

Clinical data were collected 2 years before in pre-N, monthly in on-N, and in post-N for a 1-year follow-up. The time to the first relapse event (FRE) was analyzed with Cox regression and Kaplan–Meier method. The annualized relapse rate (ARR) was calculated by dividing the total number of relapses by the total number of person-years at each time period [10]. Statistical test applied were Friedman, Mann–Whitney and Kruskal–Wallis tests. ANOVA and Wilcoxon tests were applied to make group comparisons.

Magnetic resonance imaging (MRI)

This is an observational and retrospective study; thus, MRI frequency is not regularly scheduled as in clinical trials. We are aware of the limitations of these data, consequently we did only a qualitative evaluation (new lesions yes/no, new Gd lesions yes/no), analyzed by Mc Nemar test. The radiological data considered were: presence of new T2 lesions and Gd+ T1 lesions at brain MRI scans collected in pre-N, every 3–6 months in on-N, and in post-N. The first MRI lesion event was evaluated by survival analysis and with Mantel-Cox and Kaplan–Meier methods.

Disease-free (DF) patients in post-N

Patients classified as DF were all patients who did not show relapses or variations on MRI (new or Gd+ lesions) in post-N. A multivariate analysis performed by a stepwise logistic regression (forward/backward) procedure evaluated the patient's status (DF, not DF) as the dependent dichotomic variable, while the independent variables were: age at the beginning of N, gender, age at onset of MS, number of relapses in pre-N ($n \geq 2$ versus 0–1), pre-N EDSS score, new MRI lesions (Yes, No), disease duration and the number of N infusions.

Post-N treatments

In post-N, 19 patients received a pulsed steroids treatment [11] (methylprednisolone 1 g every 15 days for 3 months), while 35 did not.

After N suspension, 12 patients (23 %) refused any treatment, although 42 patients (77 %) received an alternative DMD. 24 patients restarted N after a mean wash out (WO) of 4 months. Some of these patients restarted N because they did a "drug holiday", as suggested [12] in the past, however, some other patients restarted N because they had already been treated with first line DMD unsuccessfully, and because the introduction of Stratify test [13] added extra knowledge about patient risk profile. We are aware that this group represents 44 % of our population, we decided to consider post-N disease activity even after patients restarted N, because we focused on the effect of N suspension and on the best strategies to prevent eventual MS reactivations.

18 patients received an alternative treatment (IT) after a mean WO of 3 months: 11 patients received immunomodulants, 5 immunosuppressive treatment, and 2 patients

received Fingolimod. In our analysis, no significant differences have been found across the three groups in ARR or in EDSS (in pre-N or in on-N).

Results

We included 54 patients, 17 (31.5 %) males, 37 (68.5 %) females; 43 (79.6 %) patients were classified as criterion-A AIFA and 11 (20.4 %) as criterion-B AIFA [9]. The mean age of onset of MS was 24.6 (SD ± 6.48, Min 14, Max 51). No differences between males and females were reported. The mean time of treatment was 21 months and mean time of wash out 4 months.

Clinical disease activity

In post-N the overall proportion of patients who experienced relapses was 57.4 %. Relapses occurred on average at 6.46 months (Min 1, Max 11).

First relapse event (FRE)

Pre-N time of relapse absence was 0.48 years (5.8 months SE = 0.046), it was 0.93 in on-N (11.2 months, SE = 0.028), and 0.67 years (8.0 months, SE = 0.045) in post-N (Fig. 1). The cumulative FRE (pre-N, on-N, post-N) was different ($p = 0.0000$).

ARR

Pre-N ARR was 1.74 (SD 1.18, Min 0, Max 6). Note that Min 0 refers to only 1 patient who satisfied the criteria at the time of registration in the AIFA Register for severe relapses and highly active MRI lesions, but the first dosing was delayed by several months, because of the patient's personal reasons.

On-N ARR was 0.21 (SD 0.51, Min 0, Max 2) after 1 year and 0.22 (SD 0.49, Min 0, Max 2.13) after 2 years of treatment with N. Post-N ARR was 0.22 in the first 3 months after suspension, it was 0.99 (SD of 1.13, Min 0, Max 4) between 6 and 9 months, and 0.94 (SD 1.04, Min 0, Max 4) at 1 year (Fig. 2). The differences among pre-N, on-N and post-N ARR were statistically significant (Friedman test, $p = 0.000$). The multiple comparison test showed that ARR significantly decreased in on-N compared to pre-N ($p = 0.0000$), and raised after suspension, as post-N ARR was significantly higher than on-N ($p = 0.0004$), but significantly lower than pre-N ($p = 0.0053$).

EDSS

Median pre-N EDSS score was 3.00, on-N score was 2.25 and post-N was 2.75. The comparison between EDSS data was significant ($p = 0.047$). The multiple comparisons showed that on-N EDSS was significantly lower than pre-N ($p = 0.024$), post-N was significantly higher than on-N ($p = 0.044$), while no significant difference appeared between pre-N and post-N EDSS ($p = 0.80$).

MRI disease activity

The patients with MRI disease activity in pre-N were, respectively, 31 (58 %) in terms of Gd+ lesions and 34 (73.9 %) in terms of new T2 lesions. During on-N, 1 patient (2 %) showed Gd+ lesions, and 6 (11 %) had new lesions. In

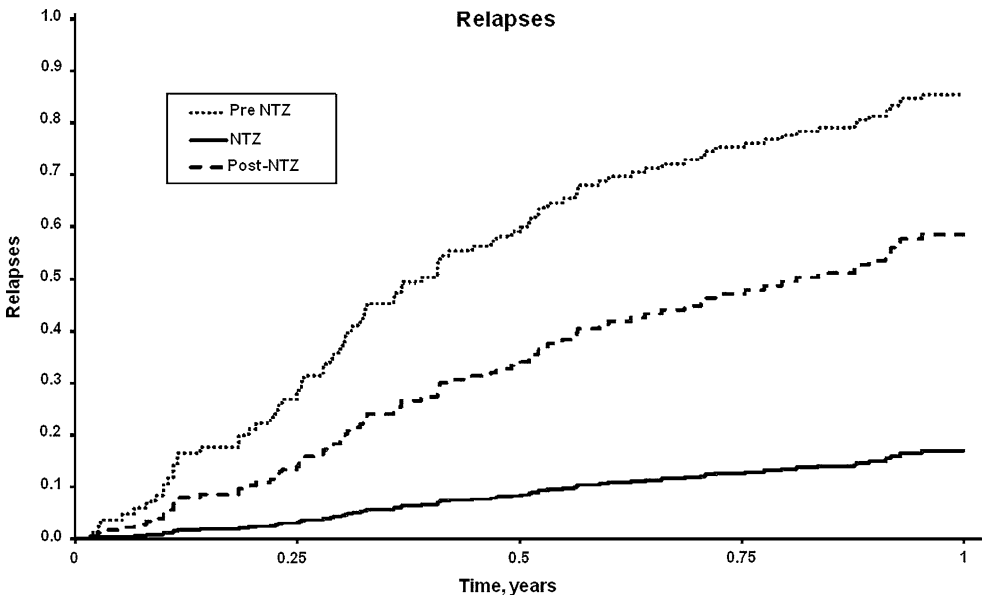

Fig. 1 *RELAPSES* Kaplan–Maier survival analysis: mean time of relapse absence of 0.48 years (5.8 month SE = 0.046) in pre-N, 0.93 years (11.2 months, SE = 0.028) on-N, while in post-N it was 0.67 years (8.0 months, ES = 0.045). The cumulative FRE collected in the 3 periods (pre-N, on-N, post-N) was different ($p = 0.0000$). Product Limit—Cox Model

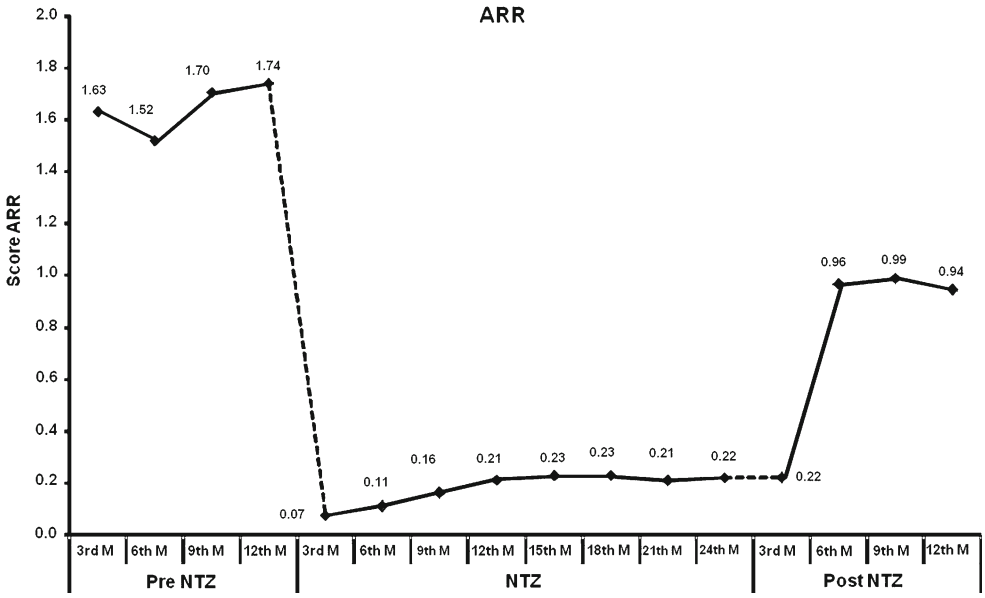

Fig. 2 *ARR* Comparison between the mean value of ARR pre-N and at the end of N calculated with Friedman's non parametric test ($p = 0.0000$) and with multiple comparisons test

Friedman Test :	Mean Value	SD	Min	Median	Max	N	P
Pre N	1.74	1.18	0.00	2.00	6.00	54	0.0000
On-N	0.21	0.51	0.00	0.00	2.00	54	
Post N	0.94	1.04	0.00	1.00	4.00	54	

Multiple comparisons test:		P
Pre-N	on-N	0.0000
	Post N	0.0053
on-N	Post N	0.0004

post-N, 24 (47.1 %) showed Gd+ enhancement and 27 (52.9 %) showed new lesions. Differences between on-N and pre-N were significant (new lesions: $p = 0.0000$; Gd+: $p = 0.0000$), and differences between on-N and post-N were significant as well (new lesions $p = 0.0001$, Gd+ lesions $p = 0.0000$). Comparing post-N and pre-N only new lesions analysis showed significant differences ($p = 0.014$). New MRI lesions occurred on average at 7 months after suspension (Min. 1, Max 12), while new Gd+ lesions occurred on average after 6.5 months (Min 3, max 12).

In post-N, 37 patients had MRI scans in the first 5 months, 31 between 5 and 8 months, and 32 patients had MRI scans evaluated after 8 months (Fig. 3a, b) (no significant differences between the 3 different periods of time, data not shown).

On-N mean time of freedom from new T2 lesions was 2.22 years (SE = 0.091) and post-N was 0.81 years (SE = 0.064) ($p = 0.0000$). New Gd+ on-N was 2.38 years (SE = 0.043) and post-N was 0.86 years (SE = 0.069) ($p = 0.0000$) (Fig. 4a, b).

Disease-free

The DF patients in post-N were 16 (29.6 %): 7 (12.9 %) were retreated with N, 5 (9.3 %) were treated with IT, 4 (7.4 %) were not treated with any treatment. None of the independent variables showed a significant effect on the disease activity (data not shown).

Post-N treatments

For patients treated with steroids, post-N ARR was 0.61 (SD 1.22, Min 0 Max 3.8); for patients not treated with steroids post-N ARR was 0.83 ($p = 0.5307$, not significant).

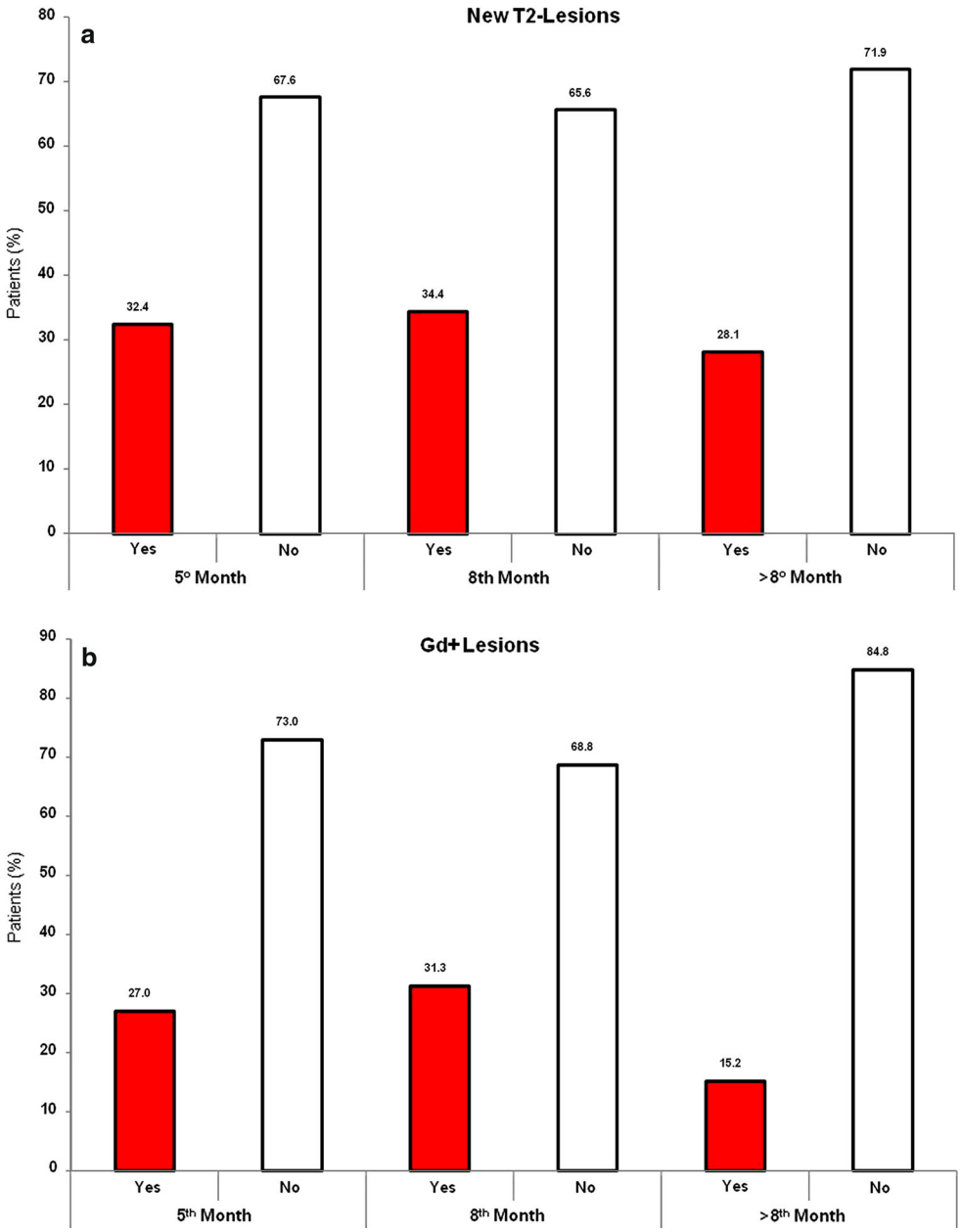

Fig. 3 *MRI* Rate of patients who developed MRI disease activity, new lesions (**a**) and new Gd+ lesions (**b**) in post-N in the different periods of time: 5 months after suspension (5th month), between 5 and 8 months after suspension (8th month), more than 8 months after suspension (>8th month)

Multiple comparisons test showed that on-N ARR was significantly lower than pre-N in all the groups of patients (IT, retreated with N and not treated) (Table 1).

In patients not treated with any IT, pre-N ARR was 2.17, on-N ARR was 0.38, post-N ARR was 1.17 ($p = 0.0058$). The multiple comparisons test showed significant differences between on-N and post-N conditions ($p = 0.0414$), but no differences between pre-N and post-N ($p = 0.2627$).

In patients treated with IT, pre-N ARR was 1.39, on-N ARR was 0.06 and post-N ARR was 0.94 ($p = 0.0033$). The multiple comparisons test showed significant differences between on-N and post-N ($p = 0.03$) but showed no differences between pre-N and post-N (0.2420) (Table 1).

In patients retreated with N, pre-N ARR was 1.79, on-N ARR was 0.25 and post-N ARR was 0.83 ($p = 0.0001$). The test for multiple comparisons showed that the difference between on-N and post-N was not significant and that post-N ARR was significantly lower in this group than pre-ARR (0.0173) (Table 1).

Post-N EDSS at 1 year in the 3 groups was compared, the differences were not significant ($p = 0.42$).

The Wilcoxon multiple comparison test showed that in patients who received IT, post-N EDSS was significantly higher than on-N EDSS ($p = 0.015$), though, in those patients who were retreated with N and in patients who did no other treatment, the differences between on-N and post-N EDSS were not significant (data not shown).

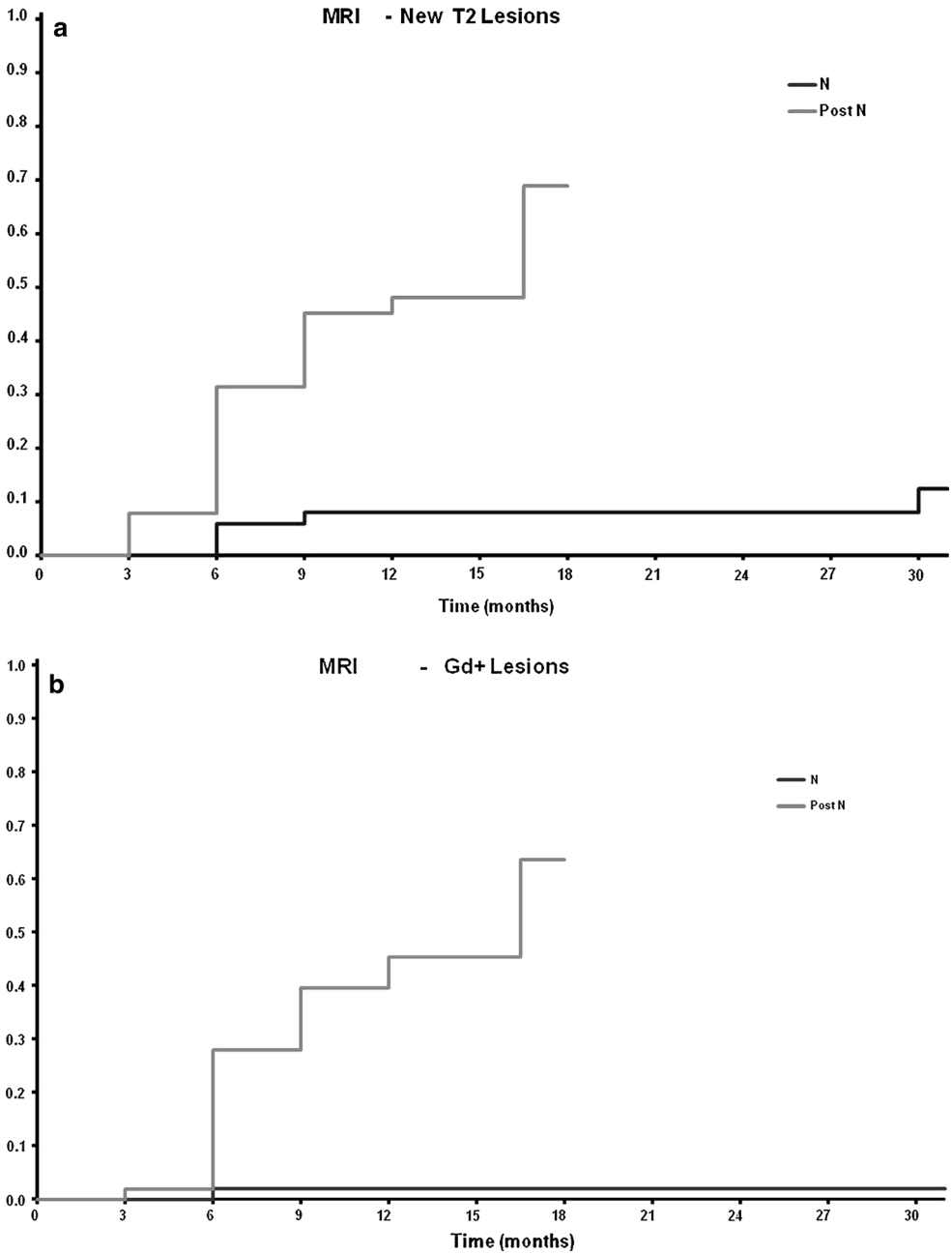

Fig. 4 *MRI* Mean time of freedom from new T2 lesions (**a**) and from new Gd+ (**b**): comparison between on-N and post-N period of time ($p = 0.0000$) for T2 lesion ($p = 0.0000$) for Gd+ lesions. On-N mean time of freedom from new T2 lesions was 2.22 years (SE = 0.091) and post-N was 0.81 years (SE = 0.064). New Gd+ on-N was 2.38 years (SE = 0.043) and post-N was 0.86 years (SE = 0.069)

Discussion

Despite the clear evidence of its efficacy [2], some true open questions about N still remain: whether N discontinuation can cause either the return of MS disease activity at baseline levels or a rebound effect, and what are the strategies that could stabilize its efficacy after suspension.

We reported the clinical and radiological data of 54 patients who discontinued N treatment, to observe the effects of suspension in a "real world" clinical setting.

Our results showed an important efficacy of N: our patients had an 88 % reduction of ARR during treatment (clinical trials 68 % reduction) [1]. However, we observed that MS activity returned after suspension of N and mean post-N ARR (0.94) was much higher than reported from clinical trials patients [10] and post-N ARR did exceed the rates observed in placebo-treated patients in the AFFIRM (0.73) and SENTINEL (0.75) trials. On the other hand, data from recent reviews never raised over this target [10].

The possibility of a rebound effect was observed since the first clinical trials [14], however, pre-treatment patients' conditions were not always evaluated [15, 16]. The knowledge of baseline patient features are very useful to better understand the high post-N disease activity of our

Table 1 Multiple comparisons test of mean ARR in the 3 different periods of time (pre-N, on-N, post-N) in: (a) patients not treated with any immunomodulatory/immunosuppressive treatment (No-IT) after suspension, (b) patients treated with immunomodulatory/immunosuppressive treatment (IT), (c) patients retreated with natalizumab (N)

		p
(a) No-IT		
Pre-N	On-N	0.0016
Pre-N	Post-N	0.2627
On-N	Post-N	0.0414
(b) IT		
Pre-N	On-N	0.0009
Pre-N	Post-N	0.2420
On-N	Post-N	0.0300
(c) N		
Pre-N	On-N	0.0000
Pre-N	Post-N	0.0173
On-N	Post-N	0.0512

cohort: our patients were younger (33.9 years old) than the clinical trial populations (40.2 years old) [10], and they showed a higher degree of disease activity (pre-N EDSS 3.00 and pre-N ARR 1.74). This might be explained by the differences between trial-selected patients and "real world" patients from clinical practice.

The study of O'Connor et al. [10] did not show evidence of rebound, the disease activity returned after suspension but the rate of patients experiencing relapses never raised over clinical-trial placebo group results (40 %). Accordingly, these authors compared their results with the placebo group. In our cohort, there were 57.4 % of patients experiencing relapses, a higher rate than the placebo patients in clinical trials (40 %) [1], and even higher than post-N interruption in other studies (around 20 %) [10]. Furthermore, as we believe that pre-N disease activity has to be considered to correctly evaluate the effects of N suspension, in our cohort we made an indirect comparison of the disease activity between before and after treatment conditions. We observed that disease activity never raised over pre-N levels, neither post-N ARR nor post-N EDSS.

Consequently, on the whole we did not observe a generalized rebound effect, but our findings were consistent with a return of the disease activity, as we experienced a very dramatic MS reactivation in a few patients, as already demonstrated in single case reports [17].

An alternative drug coverage during N interruption need to be considered. Some authors analyzed the effects of a monthly steroids treatment and of immunomodulatory therapies in post-N, but no satisfying results have been found [11, 18].

Though our sample size was too small to compare different therapeutic strategies, we observed that relapse activity increased during N interruption, regardless of whether or not patients were treated with alternative treatments.

Post-N ARR was significantly higher than on-N except for patients retreated with N, indicating that suspension did not substantially modify N effectiveness. Although in patients retreated with N post-N ARR (0.83) was significantly lower than in the pre-N (1.79), the majority of these patients (71 %) reactivated, suggesting that a "drug holyday" is detrimental. In order to evaluate the effect of N suspension it is suitable to consider the different inclusion criteria among several countries, for example the features of Italian patients are very similar to the "highly active disease" patients that in the previous reviews showed the worst disease reactivation after N suspension [10].

Despite the limitations of the present study, which is a retrospective and observational one, all data are consistent with the very good effectiveness of N applied in a clinical "real world" setting. A firm conclusion about the best therapeutic option in post-N cannot be drawn by our study. Yet, the high frequency of disease reappearance in our cohort suggests that patients after N suspension should be treated considering their previous therapeutic and clinical history. Nowadays further studies about the effects of N suspension are still warranted to prevent the loss of N beneficial effects, one of the most efficient drug in the treatment of MS.

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Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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