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# A multicenter, non-interventional study to evaluate the disease activity in Multiple Sclerosis after withdrawal of Natalizumab in Portugal

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ABSTRACT

*Objectives:* Natalizumab (NTZ) is very effective for treatment of relapsing-remitting multiple sclerosis (RRMS), its use is mainly limited by safety issues. Discontinuation of NTZ is associated with recurrence of disease activity (reactivation and rebound). The best strategy for subsequent therapy and the predictive factors for recurrence in such patients are areas of active research. We aimed to evaluate predictors of reactivation in a multicentric study.

*Patients and methods:* Multicentric retrospective observational study in five portuguese MS referral centers. Demographic, clinical and imagiological data were collected in the year prior, during and in the year following NTZ discontinuation. Predictors of reactivation and rebound after NTZ suspension were studied using a multivariate Cox model.

*Results:* Sixty-nine patients were included. They were mainly non-naïve patients (97%), with a mean age of 29.1 ± 8.3 years at diagnosis, and a mean age of 37.2 ± 10.3 years at NTZ initiation. The mean annualized relapse rate (ARR) previous, during and after NTZ was 1.6 ± 1.2, 0.2 ± 0.5 and 0.6 ± 1.0, respectively. The median EDSS before, during and after NTZ was 3.5 (IQR 3.3), 3.5 (IQR 3.5) and 4.0 (IQR 3.8), respectively. The median number of infusions was 26.0 (IQR 12.5) and the main reason to NTZ discontinuation was progressive multifocal leukoencephalopathy (PML) risk (70%). After NTZ suspension, reactivation was observed in 25 (36%) patients after a median time of 20.0 (IQR 29.0) weeks. Reactivation predictors in our sample included NTZ suspension for reasons other than PML (adjusted HR = 0.228, 95% CI [0.084- 0.616], p = 0.004), ARR before NTZ (adjusted HR = 1.914 95% [CI 1.330–2.754], p < 0.001) and a longer disease duration at time of NTZ initiation (adjusted HR = 1.154, 95% CI [1.020–1.306], p = 0.023). Rebound occurred in 5 (7%) patients after a median time of 20 (IQR 34.5) weeks.

*Conclusion:* Significant predictors of disease reactivation in our cohort were discontinuation of NTZ for reasons other than PML risk, higher disease activity before NTZ treatment, and longer disease duration. Our study provides valuable data of portuguese patients after NTZ withdrawal.

## 1. Introduction

Natalizumab (NTZ) is a humanized recombinant monoclonal antibody that binds to the α4-integrin component of Very Late Antigen-4 (VLA-4) on lymphocyte's surface, thereby inhibiting their migration across the blood-brain barrier [1].

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Despite its high efficacy, the major limitation to NTZ long-term use is the risk of developing PML, an opportunistic viral infection of the brain caused by the John Cunningham virus (JCV) [2]. Other limiting issues include treatment failure, adverse events and pregnancy [2].

In addition, NTZ suspension has been associated with disease reactivation in 20–70% of patients usually in the first 7 months [2–20]. In up to 39% of those, a rebound phenomenon (disease activity that exceeds pre-treatment levels or a severe relapse with sustained disability progression) has been observed [2–21]. In previous studies, risk factors identified for disease reactivation after NTZ suspension were variable, and included: disease aggressiveness as measured by clinical and imagiological disease activity pre- and during NTZ [4–8,12,14,15,19,20,23],age [4,5,11], duration of NTZ treatment [7,17], presence of antibodies against NTZ [8] and washout period [7,9,19,23]. It is suggested that an alternative disease modifying drug (DMD) should be promptly started to reduce the recurrence of disease activity [2,22,23] but evidence upon optimal treatment strategy is limited and controversial.

In Portugal, accordingly to EMA indication, NTZ is approved for use in naïve patients with highly active RRMS or as a second line treatment for patients in whom first line treatments have failed to control the disease. Portuguese MS patients were found to have a higher serum prevalence of JCV antibodies compared to the global population, which can make them more prone to be classified as high risk PML patients and more likely to suspend NTZ therapy [25]. Previous studies done in Portuguese RRMS patients treated with NTZ have confirmed its high efficacy [26,27], however, the period after NTZ withdrawal, the occurrence of reactivation or rebound of disease activity and their determinants have never been studied in the Portuguese population.

Our primary objective was to analyze in a population diagnosed with RRMS treated with NTZ, the influence of baseline prognostic factors on the risk for developing a reactivation or rebound of disease activity in the year following its suspension. As secondary objectives, we intended to analyze the evolution of clinical and radiological parameters before, during and after NTZ treatment.

## 2. Methods

### 2.1. Study design and settings

This hospital-based retrospective observational cohort study was conducted in five Portuguese MS referral centers (two centers at Lisbon, two at Oporto and one at Amadora). Patients undergoing treatment at the study centers who were able to sign an informed consent and fulfilling inclusion criteria were consecutively enrolled between September and December 2016. Ethical approval, according to local centers' ethical board rules, was obtained previously to study initiation.

### 2.2. Participants

The cohort comprised adult RRMS patients diagnosed according to 2001 or 2010 McDonald criteria [28,29]. Included patients were exposed to at least 6 consecutive NTZ infusions after October 2007 and discontinued NTZ treatment between March 2008 and August 2015. Patients were evaluated at least bi-annually. MRI was performed in accordance with physician discretion and its unavailability was not considered an exclusion criteria. MRI acquisition parameters were defined at each center according to local neurorradiologist preference and one of the centers had a specific protocol for MS patient's scans. Brain MRI was universally performed in initial and follow-up scans and spinal cord MRI was performed according to physician decision. Every follow-up MRI was compared to previous scans. We analyzed the annualized new T2 lesions and the number of gadolinium enhancing lesions at each period.

### 2.3. Outcome measures and statistical analysis

We defined three time-frames in respect to NTZ treatment: before NTZ (the year prior to NTZ start), during NTZ (period under NTZ), after NTZ (the year following NTZ suspension).Demographic variables collected included gender, age at diagnosis and age at NTZ start. Clinical variables collected included disease duration, number and type of DMDs prior to NTZ start, reason to discontinue NTZ (PML risk, pregnancy, inefficacy, adverse events, neutralizing antibodies, cancer and patient request), number of NTZ infusions, washout period (in weeks), annualized relapse rate (ARR) before, during and after NTZ, Expanded Disability Status Scale (EDSS) before, during and after NTZ, use of steroids during washout, DMDs used after NTZ suspension, occurrence of reactivation and time in weeks until reactivation, occurrence of rebound and time in weeks until rebound. Reactivation was defined as the recurrence of any disease activity measured by individual ARR higher than 0; rebound was defined as a severe recurrence of disease activity indicated by a higher ARR after discontinuation of NTZ compared to pre-NTZ levels or by a severe relapse defined by an increase of at least 3 points in EDSS. The PML risk was individually defined by the treating physician for each patient. Generally it was defined by the combination of positivity for JCV antibodies (especially if high titres, eg $> 0.9$), the duration of natalizumab treatment (eg, $> 2$ years) and any previous immunosuppressant medication.

Regarding the imagiological analysis, we selected all the patients who had at least one MRI in each of the periods previously defined. We analyzed the annualized new T2 lesions and the number of gadolinium enhancing lesions at each period.

Quantitative variables were summarized by mean value and standard deviation or median and interquartile range. Qualitative variables were presented in percentages and absolute number. Occurrence of reactivation and rebound, use of steroid during washout were studied as binary variables. DMDs used prior to NTZ start and DMDs used after NTZ suspension were analyzed as categorical variables with more than 2 categories in the descriptive analysis and were dichotomized as use of a second line/other DMD in the comparisons between groups and cox regression. Reason to NTZ suspension was evaluated as a categorical variable with more than 2 categories in the descriptive analysis and was dichotomized as due to PML risk/other reason in the comparisons between groups and cox regression, in order to reduce groups due to the small number of patients in categories other than discontinuation due to PML risk.

For all patients, we compared the EDSS and ARR at three time periods: the year before the initiation of NTZ treatment, during NTZ treatment and the year after its discontinuation. Comparisons between clinical and imagiological parameters during each period were performed using a Wilcoxon test.

We compared the population with complete and incomplete imagiological data using a chi-square or Mann-Whitney test in order to evaluate if the subgroups presented different demographic, clinical and radiologic characteristics.

We performed a multivariate cox regression to identify demographic, clinical and imagiological predictors of reactivation and rebound after NTZ suspension. The survival time was defined as the time, in weeks, between NTZ suspension and the occurrence of the event of interest for patients presenting reactivation/rebound during the after NTZ period or the entire period after NTZ period (52 weeks) for patients without reactivation/rebound. Potential predictors analyzed included: gender, age at NTZ treatment initiation, disease duration at NTZ treatment initiation, ARR before and during NTZ treatment, EDSS before and during NTZ treatment, duration of NTZ treatment, washout period, use of steroids during washout period, reason to stop NTZ treatment and treatment after NTZ.

Statistical analyses were performed using IBM SPSS Statistics® version 23.0 (SPSS Inc., Chicago, IL, USA) and statistical significance defined as $p < 0.05$.

**Table 1**
Demographic and clinical characteristics of patients discontinuing NTZ treatment.

| Variables | Total cohort (n = 69) | Patients with incomplete imagiological data (n = 15) | Patients with complete imagiological data (n = 54) | p-value |
|---|---|---|---|---|
| Female gender, n (%) | 45 (65.2) | 11 (73.3) | 34 (63.0) | 0.5[1] |
| Age at MS diagnosis, mean (SD) | 29.1 (8.3) | 28.7 (8.5) | 29.3 (8.4) | 0.7[3] |
| Age at NTZ start, mean (SD) | 37.2 (10.3) | 37.7 (11.1) | 37.1 (10.2) | 0.8[3] |
| Number of previous DMD, mean (SD) | 1.5 (0.7) | 1.5 (0.5) | 1.5 (0.8) | 0.9[3] |
| Patients under a second-line DMD prior to NTZ, n (%) | 2 (2.9) | 0(0.0) | 2(3.7) | 0.6[2] |
| Treatment duration before natalizumab, mean (SD) | 6.5 (3.7) | 8.7 (5.8) | 8.3 (5.6) | 0.7[3] |
| Patients interrupting NTZ due to PML risk, n (%) | 48 (69.6) | 12 (80.0) | 36 (66.7) | 0.5[1] |
| ARR, mean (SD) | | | | |
| - before NTZ | 1.6 (1.2) | 1.4 (1.5) | 1.7 (1.0) | 0.1[3] |
| - during NTZ | 0.2 (0.5) | 0.1 (0.3) | 0.3 (0.6) | 0.5[3] |
| - after NTZ | 0.6 (1.0) | 0.1 (0.4) | 0.7 (1.1) | **0.03**[3] |
| EDSS, median (IQR) | | | | |
| - before NTZ | 3.5 (3.3) | 3.5 (2.5) | 4.0 (3.0) | 0.2[3] |
| - during NTZ | 3.5 (3.5) | 2.5 (4.0) | 3.8 (3.0) | 0.5[3] |
| - after NTZ | 4.0 (3.8) | 2.5 (2.5) | 4.0 (4.0) | 0.08[3] |
| Number of NTZ infusions, median (IQR) | 26.0 (12.5) | 24.0 (20.0) | 27 (11.5) | 0.3[3] |
| Washout period, mean (SD) | 21.8 (19.3) | 20.5 (21.1) | 22.2 (19.0) | 0.5[3] |
| Patients under steroids during washout, n (%) | 22 (31.9) | 3 (20.0) | 19 (35.2) | 0.4[1] |
| Patients under a second-line DMD after NTZ, n (%) | 54 (78.3) | 13 (86.7) | 41 (75.9) | 0.5[1] |
| Patients with reactivation after NTZ suspension, n (%) | 25 (36.2) | 2 (13.3) | 23 (42.6) | 0.06[1] |
| Patients with rebound after NTZ suspension, n (%) | 5 (7.2) | 0(0.0) | 5(9.3) | 0.6[1] |

ARR: annualized relapse rate, DMD: Disease-modifying drug; EDSS: Expanded disability status score; IQR: interquartile range; MS: multiple sclerosis; NTZ: natalizumab; PML: progressive multifocal leukoencephalopathy; SD: standard deviation.
p-values are for between-group comparisons with [1] Fisher's exact test [2] Pearson Chi- Square [3] Mann-Whitney test.

## 3. Results

Sixty-nine patients from 5 Portuguese tertiary centers were included in the clinical analysis. Patients were diagnosed according to the McDonald criteria defined at the time of diagnosis, i.e. 2001 or 2010 criteria. Hence, 54 patients were diagnosed according to the 2001 criteria, and the remaining 15 patients were diagnosed according to the 2010 criteria.

Patients were diagnosed at the mean age of 29.1( ± 8.3) years and started NTZ treatment at 37.2 ± 10.3 years. In 2 (3%) patients NTZ was the first DMD. The remaining patients had been under DMD for a mean 6.5 ( ± 3.7) years before starting NTZ; most of them (54%) had been previously treated with only 1 drug, 37% with 2 drugs, 8% with 3 drugs and only 2% with 4 drugs. The majority of the non-naïve patients (97%) was previously under a first line DMD: 22 (32.8%%) glatiramer acetate and 43 (64.2%%) interferon beta formulation; 1 patient fingolimod (1.5%%) and one patient mitoxantrone (1.5%%).

Median EDSS and mean ARR before and during NTZ are presented in Table 1. The ARR during NTZ declined significantly in respect to pre-NTZ period ($p < 0.001$), while no differences were observed between EDSS before and during NTZ treatment ($p = 0.284$).

In the subgroup of 54 patients with complete imagiological data, we found that the ARR after NTZ was higher than in the remaining sample (0.7 vs 0.1, $p = 0.03$ respectively), whereas in all the other clinical results, the populations did not differ significantly (Table 1). Moreover, among the population with radiologic data, we observed a mean annualized new T2 lesion of 3.1 ( ± 5.0) and a mean of 1.4 ( ± 3.5) gadolinium enhancing lesions before NTZ start. During NTZ treatment the mean annualized new T2 lesion dropped to 0.6 ( ± 1.6) ($p < 0.001$) and mean gadolinium enhancing lesions reduced to 0.0 ( ± 0.2) ($p < 0.001$).

Patients had a median of 26.0 (IQR 12.5) infusions of NTZ. The main reason to stop NTZ was the PML risk (70%), followed by inefficacy in 12% of cases, pregnancy in another 12%, adverse events in 4% of patients and neutralizing antibodies or other reasons in the remaining.

After NTZ discontinuation, the majority of patients (97%) started another treatment, according to the treating physician discretion. Among those, a large fraction of patients started fingolimod (64%), others were treated with interferon beta formulation (9%), glatiramer acetate (8%), intravenous immunoglobulin (IVIG) pulses (8%), dimethyl fumarate (3%), azathioprine (2%) and 8% of patients restarted NTZ. The washout period was variable (1–90 weeks), the median was 16.0 (IQR 13.0) with the majority (56%) of patients waiting for a period superior to 8 weeks to resume treatment. Twenty-two (32%) patients received steroid treatment during the transition.

Parameters of clinical activity and disability during the year following NTZ are presented in Table 1. We observed a significant increase in the ARR in respect to the period under NTZ ($p = 0.006$), although it was still inferior to the pre-NTZ period ($p < 0.001$) (Fig. 1). The EDSS after NTZ suspension was significantly higher than during NTZ treatment ($p = 0.003$), however no significant differences were observed in respect to the pre-NTZ period ($p = 0.085$).

In the subgroup with complete imagiological data, we observed a mean annualized new T2 lesions of 3.6 ( ± 6.8) and a mean gadolinium enhancing lesions of 1.1 ( ± 3.2) after NTZ suspension, which represented an increase in imagiological activity in comparison to the period under NTZ ($p < 0.001$ and 0.001, respectively) (Fig. 1). This imagiological activity, including new T2 lesions and mean gadolinium enhancing lesions was comparable to the period before NTZ start ($p = 0.303$ and 0.767 respectively).

We observed that after NTZ suspension, only 5 patients (7%) presented rebound, after a median time of 20 weeks (IQR 34.5) while reactivation was much more frequent and was observed in 25 patients (36%), after a median time of 20 weeks (IQR 29.0). In addition, we observed that 48% of reactivation occurred in the first 20 weeks after NTZ suspension and in the remaining patients the reactivation occurred after a median of 40 weeks, when the majority of patients were already under other DMD.

We performed a multivariate Cox regression in order to identify clinical predictors of disease reactivation. We found that a longer disease duration and a higher ARR before NTZ were associated with a higher risk of reactivation with an adjusted hazard ratio (HR) of 1.914

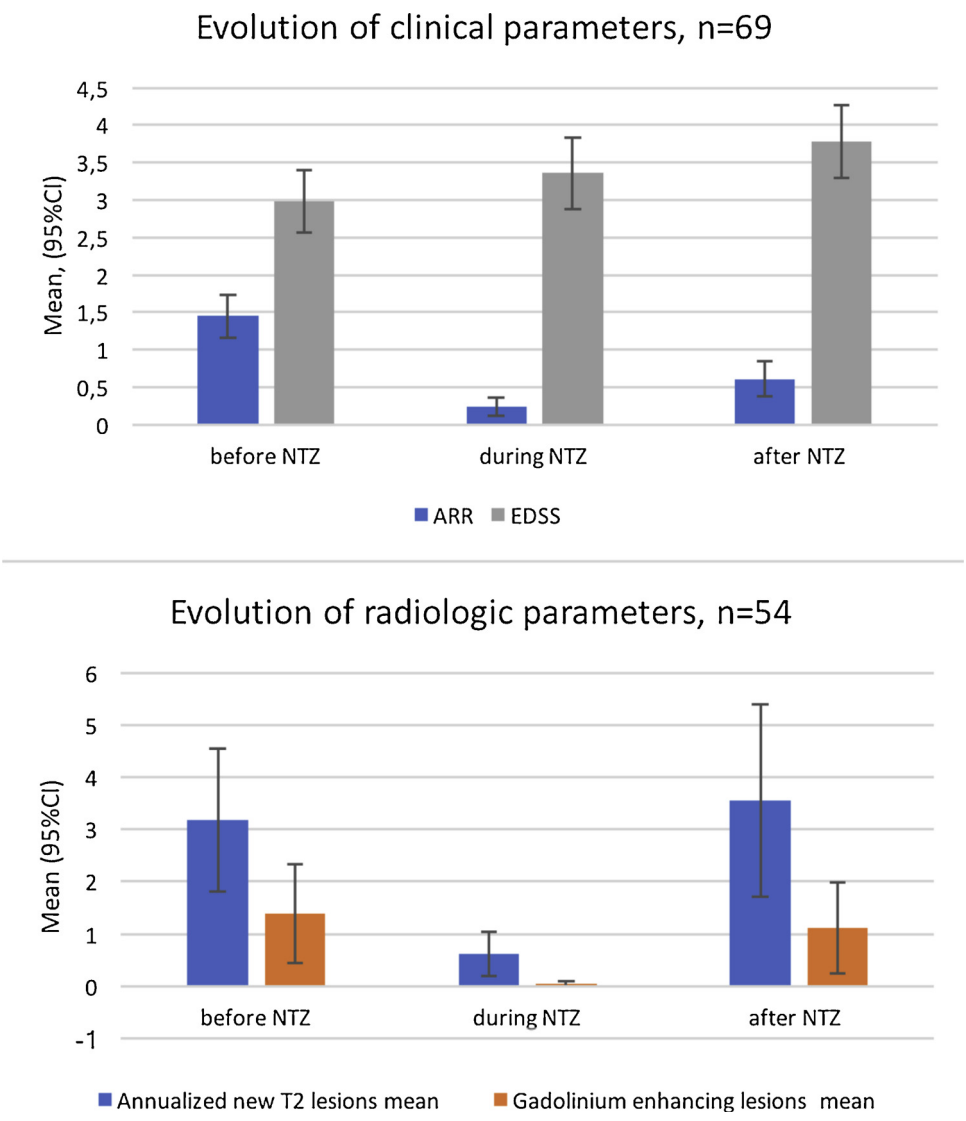


**Fig. 1.** Evolution of clinical and MRI parameters of disease activity before, during and after NTZ.

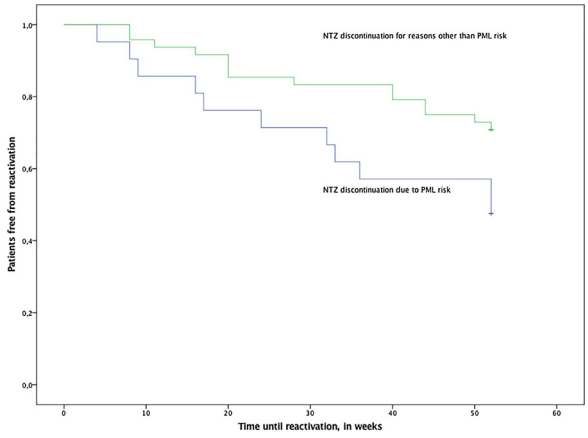


**Fig. 2.** Kaplan Meier plot of time to reactivation according to the reason to stop NTZ treatment.

(95% CI [1.330–2.754], p < 0.001) and 1.154 (95% CI [1.020–1.306], p = 0.023), respectively. On the other hand, the discontinuation of NTZ due to reasons other than PML risk was associated with a protective effect on the risk of reactivation (adjusted HR 0.228, 95% CI 0.084-

**Table 2**
Patients risk of reactivation after natalizumab suspension according to the demographic, clinical and MRI characteristics.

| | Hazard ratio | P-value | Confidence interval |
|---|---|---|---|
| Age at NTZ start | 0.954 | 0.240 | 0.883-1.032 |
| Female gender | 0.715 | 0.493 | 0.274-1.866 |
| Disease duration at NTZ start | **1.154** | **0.023** | **1.020-1.306** |
| ARR before NTZ | **1.914** | **< 0.001** | **1.330-2.754** |
| EDSS before NTZ | 0.684 | 0.107 | 0.431-1.086 |
| ARR during NTZ | 0.353 | 0.099 | 0.102-1.217 |
| EDSS during NTZ | 1.253 | 0.306 | 0.814-1.928 |
| Number of NTZ infusions | 0.991 | 0.576 | 0.962-1.022 |
| Washout period | 1.013 | 0.308 | 0.988-1.038 |
| NTZ suspension due to reasons other than PML | **0.228** | **0.004** | **0.084-0.616** |
| Use of a second-line treatment after NTZ | 1.065 | 0.902 | 0.389-2.916 |
| Use of steroid during washout | 1.169 | 0.772 | 0.407-3.356 |

ARR: annualized relapse rate, EDSS: Expanded Disability Status Scale; NTZ: Natalizumab; PML: progressive multifocal leukoencephalopathy.

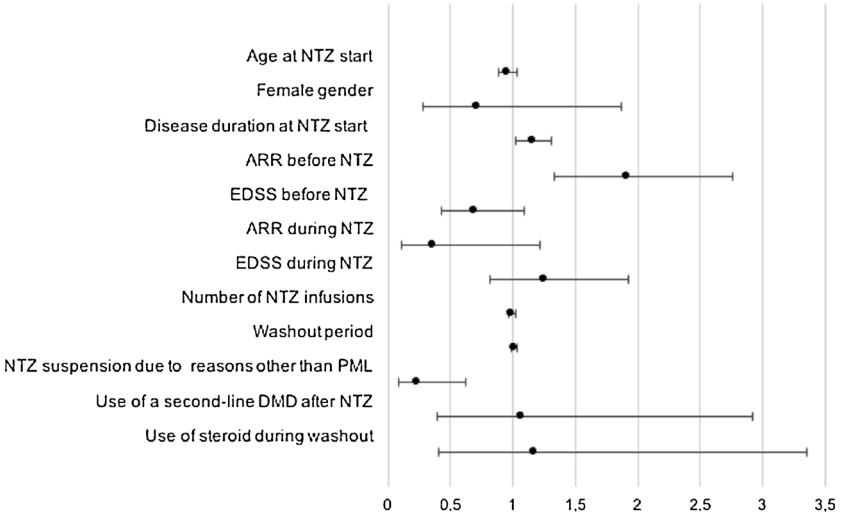


**Fig. 3.** Effect of baseline demographic, clinical and MRI characteristics on the occurrence of reactivation.

0.616, p = 0.004) (Fig. 2).The remaining clinical parameters did not influence the occurrence of reactivation after NTZ suspension (Table 2 and Fig. 3).

We performed the same analysis to find clinical parameters associated with an increase risk of rebound activity after NTZ suspension. None of the variables was a predictor of rebound.

## 4. Discussion

In our study, we found that patients treated with NTZ were mainly non-naïve, with a high ARR and a moderate degree of disability. Regarding NTZ clinical effectiveness, we observed a reduction in the ARR and a stabilization of EDSS during treatment, in accordance with previous studies conducted in our country.[24,25]. Yet, no study in the Portuguese population had evaluated MRI activity during and after NTZ treatment up to now. Hence our results are the first to confirm imagiological effectiveness of NTZ treatment in our MS population with a reduction in the new T2 and T1 enhancing lesions during NTZ treatment, with an increase after its withdrawal. High risk of PML was the main cause of NTZ suspension. After a median washout period of four months, the majority of patients restarted a DMD, mostly a second line treatment. About one third of the cohort experienced disease reactivation, after a median time of 20 weeks. In fact, after NTZ suspension, the ARR significantly increased, but not to the level observed before treatment. Imagiological activity returned to the baseline levels and the EDSS increased. Discontinuing NTZ for reasons other than PML risk, a higher disease activity before NTZ treatment, and longer disease duration were the only significant predictors that influenced the risk of reactivation.

Previous studies such as the post-hoc analysis including a total of 1866 patients, from the AFFIRM, SENTINEL, and GLANCE studies, who were observed for eight months, showed a return of disease activity, usually between 4 and 7 months, independently of receiving alternative treatment [11]. The RESTORE study, a randomized 24-week NTZ treatment interruption study, observed that up to 29% of patients after NTZ discontinuation showed an MRI disease recurrence and 15% had a clinical relapse [6]. The observational study TY-STOP, found that in the first year after NTZ cessation, up to 35% of patients had a relapse [12]. Another important observational, multicenter, French study, TYSEDMUS, which included 4055 patients, found a 45% of probability of relapse within the year after NTZ stop [4]. None of these studies reported a rebound phenomenon, however in other series its rate varied between 10% and 30% [13–19]. In our sample about one third of patients experienced reactivation, which is in accordance to the stated in the literature. The rebound rate was slightly inferior to the previous reports, 7%, this may be justified by its nonconsensual definition in the literature.

Several studies have found heterogeneous results concerning which factors might predict a higher risk of reactivation following NTZ suspension: younger age, higher disease activity before and during NTZ, higher EDSS before and during NTZ treatment [1].

In our study, we found that discontinuation of NTZ for reasons other than PML risk was the most important determinant of reactivation, reducing this risk to almost to one fifth. Since about 40% of patients in this group were pregnant (a known protective condition), this might have been responsible for a decline in disease activity and a consequent lower risk of reactivation.

Pre-NTZ clinical activity was another important clinical determinant, almost doubling the risk of reactivation. It seems reasonable that the level of disease activity before NTZ impacts on the prognosis following NTZ suspension, since the effect of the drug is only transient, and the same individual factors that drove the disease aggressiveness remain unchanged throughout the treatment, and will determine a higher risk of disease reactivation.

Finally, we observed that a higher disease duration was associated with a slight increase in the risk of reactivation which although controversial has been reported in the literature [20]. Several studies have been developed to analyze the best strategy to minimize the risk of reactivation [12]. Despite some conflicting results, switching to a first line option (AG or INF) seems to significantly increase the risk of MS reactivation, but the transition to a more effective drug like fingolimod appears to be a good option [22]. Although in our sample the risk for reactivation was not influenced by starting a second line treatment, this might be related to the small number of patients under a first line treatment which prevents us from finding a statistical difference.

The washout period is a very well-established determinant of disease activity after NTZ withdrawal [8]. This is thought to be related to the desaturation of α4-integrin receptors below the level of 80%, which seems to happen after 8 weeks of NTZ suspension [30]In our sample, the number of reactivation cases through the year following NTZ suspension presented a bimodal distribution: 32% of patients presented a reactivation after a median time of 8.5 weeks (IQR 7.0), while the remaining 68% presented a reactivation after a median of 36.0 (IQR 25.0) weeks. As such, the former might reflect a reactivation due to desaturation of the α4-integrin receptor, while the latter probably relates to the inefficacy of the subsequent DMD adopted to control disease activity. We consider that this was probably the reason why the washout period was not a predictor in our population.

Despite some conflicting results, some studies suggested that monthly methylprednisolone treatment during the washout period could determine a safer transition to another therapy [9]. This was not confirmed in our population.

This was the first Portuguese multicentric study addressing the occurrence of clinical reactivation and rebound after NTZ stop and their predictors conducted in a large sample of MS patients in a real-world setting. Nevertheless, we recognize the following limitations: the lack of controls inherent to its retrospective design, the incomplete imagiological data and the lack of uniformity between centers regarding the frequency and equipment used to perform the MRI studies. However, we observed that the group of patients with complete and incomplete data was similar in the large majority of characteristics. It is also important to highlight that our results should be carefully interpreted given the large period of data collection. It corresponded to the introduction of NTZ as the only second line treatment in Portugal, explaining why our population comprised mainly non-naive patients with a longer disease course before NTZ was ensued, approximately eight years since the diagnosis of MS.

## 5. Conclusions

In our cohort, disease reactivation occurred in 36% patients, after a median time of 20.0 weeks, and only 7% of patients experienced a rebound phenomenon. Discontinuing NTZ for reasons other than PML risk, a higher disease activity before NTZ treatment, and a longer disease duration were the only significant predictors that influenced the risk of reactivation. There is still an urgent need to find markers that could identify which patients are at higher risk of reactivation after NTZ discontinuation, so a more aggressive approach to control the disease could be adopted. Furthermore, it is also urgent to establish which is the best treatment option we should recommend to these patients, and the adequate time to introduce it, in order to have fewer and milder reactivations and ideally no rebounds.

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