Treatment of Multiple Sclerosis with Natalizumab: Experiences from a Real-Life Cohort Over 15 Years

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Abstract

Background

Natalizumab (NTZ) has been used for treatment of highly active relapsing-remitting multiple sclerosis (MS). When stopping NTZ the risk of severe rebound phenomenon has to be considered. We aimed to investigate the use of NTZ in clinical routine and focused on identification of potential risk factors for disease reactivation after treatment discontinuation.

Methods

At the Medical University of Innsbruck, Austria, we identified all MS patients who were treated with NTZ and performed a retrospective analysis on therapeutic decision making, disease course before, during and after treatment with NTZ and on risk factors for disease reactivation after NTZ discontinuation.

Results

235 NTZ treated MS patients were included, of whom 105 had discontinued treatment. At NTZ start disease duration was 5.09 (IQR 2.09-10.57) years, average number of total relapses was 4 (IQR 3-6) and median EDSS 2.0 (range 0-6.5), whereby these values significantly decreased over time. Reduction of annualized relapse rate (ARR) on treatment was 93% and EDSS remained stable in 64%. In a multivariate regression model only conversion to secondary progressive MS (SPMS) on treatment was significantly associated with lower risk of disease reactivation after NTZ, while ARR before treatment was associated with earlier disease reactivation.

Conclusions

We could confirm the high therapeutic efficacy of NTZ which trends to be used earlier in the disease course nowadays. Discontinuation of NTZ seems safe only in patients who convert to SPMS during treatment, while higher ARR before NTZ increases the risk of disease reactivation after treatment discontinuation.

Introduction

Natalizumab (NTZ) is a humanised monoclonal antibody used for treatment of highly active relapsing remitting multiple sclerosis (MS). The mechanism of action is based on blocking α4 integrin (very late antigen 4, VLA-4) on the surface of lymphocytes so that the interaction with the vascular cell adhesion molecule 1 (VCAM-1) and the consecutive migration of autoreactive lymphocytes through the blood brain barrier is reduced [1]. The safety and efficacy of NTZ was approved in two large randomised, controlled studies (AFFIRM, SENTINEL) showing a reduction of annualised relapse rate of about 70% [2-3]. NTZ was finally approved by the US Food and Drug Administration (FDA) as well as in the European Union in 2006 [1]. From this time NTZ has been increasingly used in patients with highly active disease course with at least two relapses within one year and according MRI findings or, mostly, in patients where other DMTs

like interferon-beta and glatirameracetate did not show sufficient therapeutic effectiveness [4]. With stratification tests for anti-JC virus (JCV) antibodies [5-6] and discovery of dependence of PML risk from treatment duration [7-8] and JCV antibody index [9] new guidelines for monitoring NTZ treated MS patients were introduced [10-11]. As new highly effective therapeutic options were approved during recent years and the risk of disease reactivation after NTZ discontinuation raised awareness [12-23] safe strategies for switching from NTZ to other DMTs have been sought. Extended interval dosing [14], shorter intervals of treatment interruption after NTZ, short term MRI monitoring during DMT switch or preference of certain DMTs have been discussed in order to avoid a rebound of the disease [15-16].

While NTZ is a well-studied drug with reliable data from clinical trials, analysis of a real-life cohort could add important insights about the use of NTZ in clinical routine, how the disease develops before, during and after NTZ treatment and identify risk factors for disease reactivation after stopping NTZ.

Results

At the Medical University of Innsbruck (MUI), Austria, from 2006 to December 2020 a total of 242 patients were treated with NTZ, all of those had a relapsing-remitting disease course at treatment start. Seven patients had to be primarily excluded because sufficient data for any analysis were not available. This resulted in a study cohort of 235 patients. Figure 1 shows the number of patients who stayed on treatment, discontinued NTZ or were lost to follow up. Of these 235 patients 181 (77.0%) were female and 54 (23.0%) male.

Pre-treatment analysis

A total of 235 patients were included for pre-treatment analysis. The median age at NTZ start was 32.8 (IQR 25.4-39.3), the median disease duration before NTZ start was 5.09 (IQR 2.09-10.57) years. Since the stratify test for anti-JCV antibodies was introduced in 2010, JCV status at treatment start was available for 196 patients excluding those 39 patients who started NTZ before the JCV testing era. Of these, 90 (45.9%) were positive for anti-JCV antibodies at treatment start. Detailed data of treatment history before NTZ were available from 232 patients and are displayed in table 1.

The median EDSS at start of NTZ was 2.0 with a range between 0 and 6.5, details are shown in Figure 2. [insert Figure 2] Before start of NTZ patients experienced a median of four relapses (IQR 3-6) from disease onset, the median annualized relapse rate (ARR) was 1.01 (IQR 0.60-1.78). Change of disease duration, EDSS score at treatment start and relapses before NTZ from introduction of NTZ in 2006 to the present is shown in Fig. 3 and revealed a significant decrease of EDSS ($p=0.002$, $r=-0.198$) and relapses ($p<0.001$, $r=-0.263$) before start of NTZ over time.

On treatment analysis

A total of 163 patients were eligible for on-treatment analysis. Mean treatment duration was 6.27 ± 3.27 years for patients who were still on treatment and had follow ups until December 2020, and 3.35 (IQR 1.50-6.76) years for patients who discontinued NTZ. In the cohort of patients with at least two years follow up the reduction of ARR was 93% ($p<0.001$, $Z=-10.82$, $r=-0.83$), the median ARR decreased from 1.03 (IQR 0.61-1.73) to 0 (IQR 0-0.13). Figure 4 shows change of EDSS score during the treatment period for patients who remained on NTZ for at least two years. [insert Figure 4] EDSS data from 147 patients were available. 94 (63.9%) of these showed a stable or confirmed improvement of EDSS score during the whole treatment period, whereas 42 (28.6%) experienced a confirmed EDSS progression of at least one point on treatment. The median annualized EDSS progression was 0 (IQR -0.07-0.14), there was no difference between patients who continued and discontinued NTZ regarding this analysis. From 187 patients, a follow up of anti-JC virus antibodies during treatment was available. 14 (7.5%) of these converted from initially negative to positive anti-JC virus antibody status. In the whole NTZ cohort three cases of PML were observed. Of all NTZ treated patients 40 received extended interval dosing (EID) at any time, i.e. extension of intervals between NTZ infusions to five to eight weeks. Median EID period was 0.82 (IQR 0.42-3.63) years and median ARR on EID 0 (IQR 0-0.15). Of the 40 EID patients only four (10%) experienced a relapse during EID and 22 discontinued NTZ thereafter.

Discontinuation analysis

In total 105 of 235 patients at MUI discontinued NTZ until December 2020. The reason for discontinuation is displayed in table 2, where positive anti-JC virus antibody status was the most common (55.2%) followed by secondary disease progression (17.1%). Most patients were switched to another DMT, most commonly to fingolimod (41.8%) followed by ocrelizumab (15.3%) and 12 (12.2%) patients who did not receive any DMT after discontinuation of NTZ. For details see table 3. In patients who were on NTZ for at least two years we analysed the EDSS progression after treatment discontinuation. For this analysis EDSS scores were available for 47 patients at one and 18 patients at five years after NTZ discontinuation (Figure 5). [insert Figure 5] After one year, 16 of 47 patients (34.0%) experienced an EDSS worsening of at least 0.5 points, while after five years this proportion was 9 of 18 (50.0%). For 59 patients, documentation of any relapses and MRI data were available. Of these, 32 (54.2%) experienced at least one relapse after NTZ discontinuation while 35 (59.3%) had MRI activity, 25 of those showed CEL as well. Finally, these 59 patients were selected for a regression model for identifying risk factors for disease reactivation. First, all clinical and demographic variables were analysed separately between patients with and without disease reactivation. Significant differences between these groups were obtained for EDSS at treatment discontinuation (Mann-Whitney-U test, $p=0.031$, $Z=-2.156$, $r=-0.28$) and conversion to SPMS (chi-square test, $p=0.005$, $\varphi=-0.37$). EID duration, EDSS at NTZ start and discontinuation as well as interval between NTZ and following DMT were not significantly associated and finally excluded because only available for part of the patients. A detailed analysis for single DMT or moderate versus highly active DMT was not expedient due to small subgroups and therefore missing statistical power. Table 4A shows results including those variables which were available for all patients. The only significant result was obtained for conversion to SPMS which showed

to be protective (OR 0.08, p=0.03) for disease reactivation after NTZ discontinuation while all other variables did not turn out as significant potential risk factors in this model. Results of Cox regression model for time to disease reactivation after NTZ are shown in table 4B and revealed annualized relapse rate before NTZ as the only significant risk factor (OR 1.46, p=0.014) for early disease reactivation after treatment discontinuation. Regarding MRI data and EDSS there were not enough data for performing similar calculations. In the univariate analyses for EDSS progression after NTZ discontinuation performed on 43 patients only treatment duration (p=0.038, Z=-2.077, r=-0.32) revealed a significant correlation.

Discussion

With this retrospective cohort analysis we aimed to describe the use of NTZ, its efficacy and the risk factors for disease reactivation after discontinuation in clinical practice outside of controlled studies. By including all available source data, gathered up to decades, we were able to generate a very complete dataset with few missing data allowing detailed analyses of clinical history over a long period. In contrast to most multi-centre registries this monocentric approach includes a large number of variables and reduces reporting bias while it limits the number of patients and, consequently, the power of some analyses. Therefore, this study has some advantage regarding studying clinical practice and individualized treatment decisions.

We present a cohort of 235 NTZ treated patients representing a typical MS cohort, predominantly female and aged about 33 years at start of NTZ [18]. The disease history before start of NTZ varied broadly between individual patients, showing a median of four relapses, five years of disease duration and a median EDSS of 2.0 at time of NTZ start. One central objective of this study was to explore change of clinical practice over time. There is a trend that NTZ as a highly effective DMT is used earlier during the disease course nowadays, as indicated by less relapses and a lower EDSS at NTZ start as compared to the early treatment era. This is in line with current expert opinions which favour early highly effective treatments in active MS patients [19-20] showing that we gradually took this path in clinical practice.

Regarding treatment efficacy our findings agree with the well-known results from controlled studies [2-3] as well as the reports from other real-life cohorts [21]. For efficacy analysis we included patients with a minimum treatment duration of two years. Since relapses on treatment with NTZ occur rarely a longer observation period is favourable for validity of data and less risk of overestimating ARR due to single relapses especially around the time of treatment change. We found a strong relative reduction of ARR by 93%, while 64% of patients had a stable or improved EDSS score during the whole treatment period similar to other observational analyses [21-22] which had comparable median treatment durations to our study. As a reference centre for the whole region of Western Austria we reported three cases of PML, whereby two of those were referred from other hospitals which explains the high proportion of PML in a relatively small cohort. As EID became an option for possible PML risk reduction [14, 23-24], 90% of a total of 40 patients who switched from standard to extended interval dosing did not experience disease

activity during the EID period. However, median EID duration was short with less than one year in this subpopulation.

Finally, we focused on those patients who discontinued NTZ in whom positive anti-JC virus antibody status after a treatment duration of two years or longer was the leading reason for NTZ discontinuation. Many patients were switched to fingolimod, probably because this has been the first option of an effective treatment beside injectables and NTZ from 2011. The switch from NTZ to fingolimod was investigated in various studies [25-26], most of them deemed this strategy relatively safe. As other highly effective drugs have been approved especially ocrelizumab became another promising option for a switch after NTZ [27], so that this drug was the second most used in our cohort after NTZ discontinuation. Different studies tried to identify risk factors for disease reactivation after stopping NTZ, with some inconsistent results [15, 28-32]. In most of these studies risk factors for disease reactivation were longer washout periods of NTZ, higher relapse rate before NTZ and – inconsistently – younger age, higher baseline EDSS before NTZ, and disease activity on NTZ measured by relapses and EDSS progression. Butzkueven et al. [28] identified shorter treatment duration as additional risk factor as well. While Lo Re et al. [30] assumed a lower risk of disease reactivation on fingolimod compared to interferon-beta, glatirameracetate and teriflunomide, Capobianco et al. [33] did not report an impact of the DMT chosen after NTZ.

In our univariate analysis conversion to SPMS and higher EDSS at NTZ discontinuation were associated which lower risk for disease reactivation, and to some extent shorter treatment duration as well. However, in the multivariate regression model performed on patients who were on treatment at least two years with measured outcome whether they had or had not a relapse after NTZ discontinuation, none of the above variables turned out as an independent risk factor except conversion to SPMS. Furthermore, annual relapse rate before NTZ was associated with earlier disease reactivation in those patients who experienced at least one relapse after NTZ discontinuation.

There may be several reasons for the above mentioned inconsistent results between different studies and for discrepancies to our study. Most of these studies identified the treatment gap between NTZ and subsequent DMT as a risk factor. Our analysis in this point may be limited by the low number of patients and, particularly, treatment gaps were usually less than three months in our cohort while other studies had a considerable number of patients with longer intervals between NTZ and following DMT which allowed them to show significant influence of this variable. Moreover, the selection of patients for multivariate analyses was different between studies. For example, Butzkueven et al. [28] only included patients who switched to an oral therapy after NTZ. Mustonen et al. [29] calculated their model case by case according to the following DMT. Salhofer-Polanyi et al. [32] analysed a cohort of patients of whom 50% had a treatment duration <2 years. Vidal-Jordana et al. [31] chose significant clinical worsening by EDSS progression after stopping NTZ for their multivariate model, which cannot be counted as immediate reactivation. In the study of Lo Re et al. [30] only in univariate analyses the described variables were significantly associated with disease activity after NTZ. Conversion to SPMS, however, was not included as a variable in all these studies. Prosperini et al. [15] included further studies in their review

finding poor evidence for any risk factor for disease reactivation which is in line with our results. Altogether, there is a large variation regarding study designs and choice of outcome variables across the different studies, which makes it hard to identify reliable risk factors.

According to our data, patients who convert to progressive MS during treatment could be more safely withdrawn from NTZ independent from further treatment strategy, while for all patients who are stable on NTZ without signs of disease progression there are no clear risk factors for post-NTZ disease activity. Patients with high annual relapse rate before NTZ seem to be at risk for earlier disease reactivation after stop of NTZ so that one should consider short time intervals to start of alternative treatment especially in those patients.

Methods

Patients and data acquisition

At MUI an electronic MS database has been established in 2004 including all patients treated at the MS outpatient clinic. This database includes data such as demographic data, details of MS course, diagnostic investigations, treatment history, EDSS and onset of secondary progression [17].

This database was used for pre-selection of patients generating a data acquisition file including all patients who were ever treated with NTZ. In order to gather as many and exact data as possible, in a second step we searched all source data, i.e. the hospital's medical records. By this, a final data file for further analyses was created.

For pre-treatment analysis all patients were included who had at least one visit after treatment start of NTZ and where treatment history such as date of diagnosis, number of relapses, disease modifying treatments (DMTs) and date of NTZ start were sufficiently recorded. On-treatment analysis was performed on patients who had a treatment duration of at least two years and were continuously followed-up every 3-6 months on treatment. The same criteria were used for discontinuation analysis. Disease reactivation after NTZ discontinuation was defined by relapses or MRI activity, i.e. new T2 or contrast-enhancing lesions (CEL).

Statistics

Statistical analysis was performed using SPSS 26.0 (IBM, Armonk, NY, USA). Shapiro-Wilk and Kolmogorov-Smirnov normality test were used simultaneously for analysing distribution of data. Descriptive data are displayed as median and interquartile range (IQR) or range as indicated or mean and standard deviation as appropriate. Depending on type and distribution of data, Mann-Whitney U, t test or Chi-square test were used for group comparison. Correlations were performed using Pearson correlation coefficient and Z-scores. Linear regression was used for dependence of EDSS at treatment start, relapses before NTZ start and disease duration at time of NTZ start (dependent variables) from date of treatment

start (independent variables). Binomial logistic regression was performed for identifying possible predictive variables for disease reactivation after NTZ discontinuation. Patients who experienced disease reactivation after discontinuation of NTZ were additionally included in a Cox regression model for identifying risk factors for early relapses after treatment cessation. Two-tailed P-values <0.05 were considered statistically significant.

Declarations

Ethical approval

The study was approved by the ethical committee of the Medical University of Innsbruck, Austria (approval number 1033/2021). Permission to take informed consent was formally waived by the approving ethical committee. In this retrospective study all analyses were performed with pseudonymised data, which did not require a written informed consent based on applicable ethical practice and the statement of the ethical committee which approved the study. All methods were performed in accordance with the relevant guidelines and regulations.

Availability of data

All data are available upon request from the corresponding author.

Authors' contribution

MA has participated in the conception and design of the study, acquisition, statistical analysis and interpretation of data, and in drafting the manuscript.

AZ has participated in conception of the study, acquisition of data and reviewing the manuscript for intellectual content.

HH has participated in acquisition of data and reviewing the manuscript for intellectual content.

GB has participated in acquisition of data and reviewing the manuscript for intellectual content.

FDP has participated in acquisition of data and reviewing the manuscript for intellectual content.

EF has participated in acquisition of data and reviewing the manuscript for intellectual content.

SW has participated in acquisition of data and reviewing the manuscript for intellectual content.

TB has participated in acquisition of data and reviewing the manuscript for intellectual content.

FD has participated in the conception and design of the study, acquisition of data, reviewing the manuscript for intellectual content and supervised the study.

Conflicts of interest

MA received speaker honoraria and/or travel grants from Biogen, Merck, Novartis and Sanofi. AZ has participated in meetings sponsored by, received speaking honoraria or travel funding from Biogen, Merck, Sanofi-Genzyme and Teva. HH has participated in meetings sponsored by, received speaker honoraria or travel funding from Bayer, Biogen, Merck, Novartis, Sanofi-Genzyme, Siemens, Teva, and received honoraria for acting as consultant for Biogen and Teva. GB has participated in meetings sponsored by, received speaker honoraria or travel funding from Biogen, Celgene, Lilly, Merck, Novartis, Roche, Sanofi-Genzyme and Teva, and received honoraria for consulting Biogen, Celgene, Roche and Teva. FDP has participated in meetings sponsored by, received honoraria (lectures, advisory boards, consultations) or travel funding from Bayer, Biogen, Celgene, Merck, Novartis, Sanofi-Genzyme, Roche and Teva. Her institution has received research grants from Roche. KB has participated in meetings sponsored by and received travel funding from Roche. EF declares that there are no conflicts of interest. SW has participated in meetings sponsored by, received honoraria or travel funding from Biogen, Merck, Novartis, Sanofi Genzyme, Teva, Allergan, Ipsen Pharma and Roche. TB has participated in meetings sponsored by and received honoraria (lectures, advisory boards, consultations) from pharmaceutical companies marketing treatments for multiple sclerosis: Almirall, Bayer, Biogen, Biologix, Bionorica, Celgene/BMS, Genzyme, GSK, MedDay, Merck, Novartis, Octapharma, Roche, Sandoz, Sanofi/Genzyme, TG Pharmaceuticals, TEVA-ratiopharm and UCB. His institution has received financial support in the last 12 months by unrestricted research grants (Biogen, Bayer, Celgene/BMS, Merck, Novartis, Roche, Sanofi/Genzyme, and TEVA ratiopharm) and for participation in clinical trials in multiple sclerosis sponsored by Alexion, Bayer, Biogen, Merck, Novartis, Octapharma, Roche, Sanofi/Genzyme, and TEVA. FD has participated in meetings sponsored by or received honoraria for acting as an advisor/speaker for Almirall, Alexion, Biogen, Celgene, Genzyme-Sanofi, Merck, Novartis Pharma, Roche, and TEVA ratiopharm. His institution has received research grants from Biogen and Genzyme Sanofi. He is section editor of the MSARD Journal (Multiple Sclerosis and Related Disorders).

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Tables

Table 1: Disease modifying treatment (DMT) before NTZ.

A

Number of DMTs before NTZ	N (%)
0	19 (8.2)
1	135 (58.2)
2	56 (24.1)
3	16 (6.9)
4	5 (2.2)
5	1 (0.4)

B

DMTs before NTZ	N (%)
IFN-beta	192 (56.6)
Glatirameracetate	59 (17.4)
IVIG	34 (10.0)
Dimethylfumarate	14 (4.1)
Fingolimod	7 (2.1)
Teriflunomide	4 (1.2)
Cyclophosphamide	3 (0.9)
Mitoxantrone	2 (0.6)
Azathioprine	2 (0.6)
Alemtuzumab	1 (0.3)
Rituximab	1 (0.3)
Ciclosporine	1 (0.3)

Legend:

A: Number of different DMTs before treatment start with NTZ in a total of 232 patients.

B: Type of DMT before treatment start with NTZ. All DMTs per patient administered before NTZ were considered, resulting in 339 DMTs in 213 patients with pre-treatment.

Abbreviations: DMT, disease modifying treatment; IFN, interferon; IVIG, intravenous immunoglobulins.

Table 2: Reason for NTZ discontinuation.

Reason for discontinuation	N (%)
JCV positive	58 (55.2)
SPMS	18 (17.1)
Disease activity	8 (7.6)
NAbs	7 (6.7)
By request of patient	6 (5.7)
Comorbidity	6 (5.7)
Pregnancy	4 (3.8)
PML	3 (2.9)
Adverse event	3 (2.9)

Legend: 105 patients discontinued NTZ. Those patients who stopped treatment due to pregnancy are planned to restart NTZ after delivery. Abbreviations: JCV positive, positive for Anti-JC-Virus antibodies; SPMS, secondary progressive multiple sclerosis; NAbs, neutralizing antibodies; PML, progressive multifocal leukoencephalopathy.

Table 3: First DMT after NTZ.

DMT after NTZ
Fingolimod
Ocrelizumab
No DMT
Glatirameracetate
Dimethylfumarate
Rituximab
Siponimod
IVIG
IFN-beta
Mitoxantrone
Cyclophosphamide

Legend: For 98 patients data about further treatment strategy after discontinuation of NTZ were available. Abbreviations: DMT, disease modifying treatment; IVIG, intravenous immunoglobulins; IFN, interferon.

Table 4: Regression models for potential risk factors of disease reactivation (A, binomial logistic regression) and early relapses (B, Cox regression) after NTZ discontinuation.

A

Variable	P-values	OR	95% CI
Gender (female)	0.216	3.63	0.47-28.03
Age	0.391	0.96	0.86-1.06
Disease duration	0.072	1.27	0.98-1.65
ARR before NTZ	0.226	1.78	0.70-4.58
Duration of NTZ treatment	0.685	0.94	0.68-1.29
Number of relapses before NTZ	0.844	1.03	0.74-1.44
Number of relapses on NTZ	0.749	0.70	0.08-6.09
ARR on NTZ	0.816	3.31	0.00-80615
EID	0.218	0.32	0.54-1.95
SPMS	0.030	0.08	0.01-0.79
Number of DMTs before NTZ	0.222	2.11	0.64-6.99

B

Variable	P-values	OR	95% CI
Gender (female)	0.098	3.15	0.81-12.24
Age	0.866	1.01	0.94-1.08
Disease duration	0.771	1.02	0.89-1.17
ARR before NTZ	0.014	1.46	1.08-1.96
Duration of NTZ treatment	0.566	1.08	0.84-1.38
Number of relapses before NTZ	0.139	0.83	0.65-1.06
Number of relapses on NTZ	0.626	1.44	0.33-6.17
ARR on NTZ	0.718	0.34	0.01-120.21
EID	0.928	0.92	0.14-6.13
SPMS	0.336	0.26	0.02-4.01
Number of DMTs before NTZ	0.135	2.17	0.79-5.98

Legend:

A: For binomial linear regression model patients who discontinued NTZ after ≥2 years of treatment duration were divided in patients with and without occurring relapses after NTZ discontinuation. This

model focused on risk factors whether there was a disease reactivation after stop of NTZ or not.

B: Patients who experienced disease reactivation were included in a Cox regression model in order to investigate potential risk factors for time to disease reactivation.

Both tables show significances (p-values) and odds ratio for each analysed potential risk factor.

Abbreviations: ARR, annualized relapse rate, EID, extended interval dosing before discontinuation; SPMS, secondary progressive multiple sclerosis; DMT, disease modifying treatment; OR, odds ratio; CI, confidence interval.

Figures

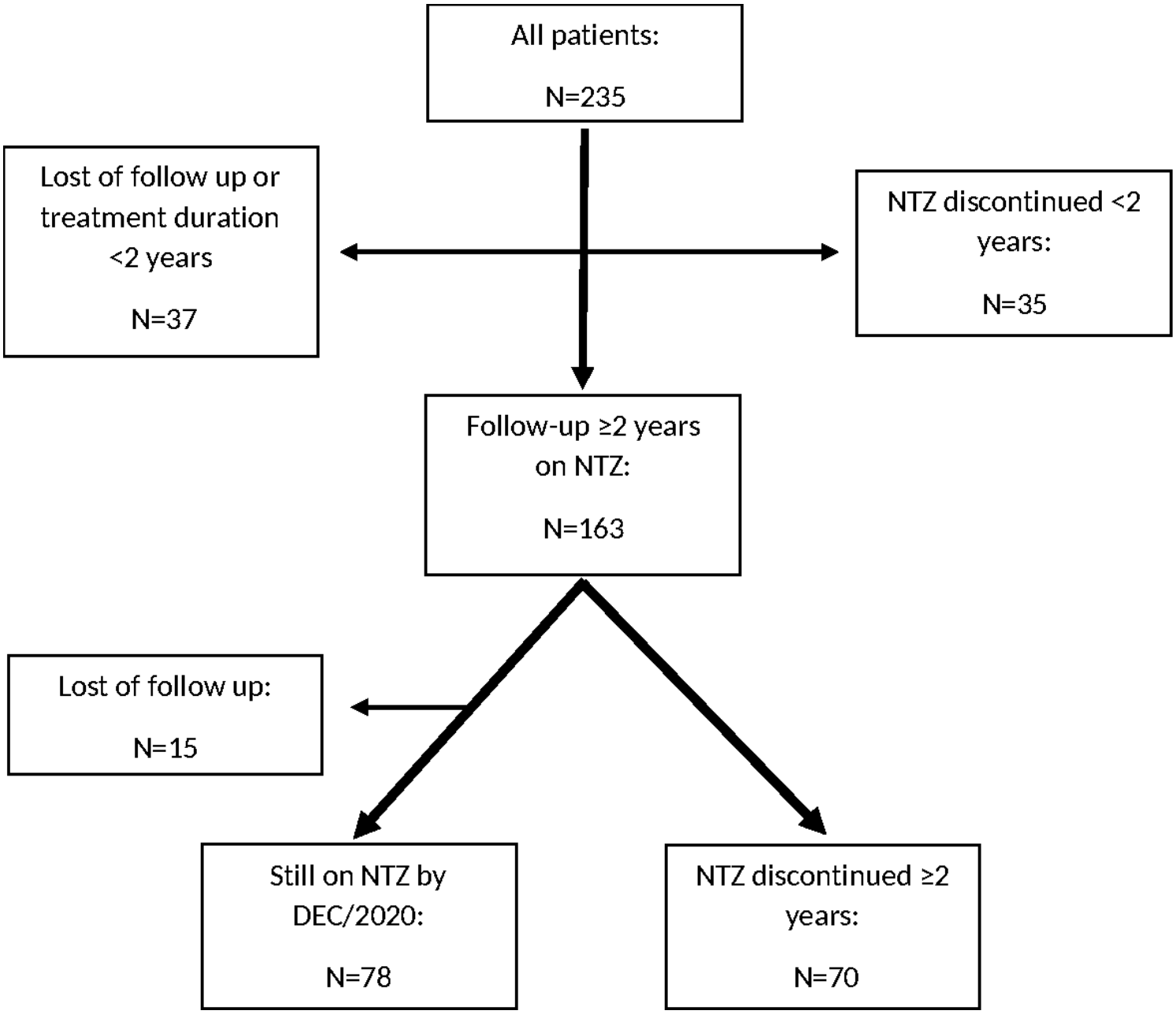

Figure 1

Cohort of patients treated with NTZ, flow chart Legend: Flow chart of all MS patients at the University hospital of Innsbruck, Austria, treated with NTZ from 2006 to 2020. All these selected patients (N=235) were eligible for the pre-treatment analysis. For the on-treatment analysis patients with a minimum treatment duration of two years were considered (N=163) by excluding those patients who were lost of follow-up or discontinued NTZ within less than two years or were still on NTZ by DEC/2020 but with a treatment duration less than two years. The discontinuation analysis was performed on all patients who discontinued NTZ before or after a treatment duration of two years (N=105).

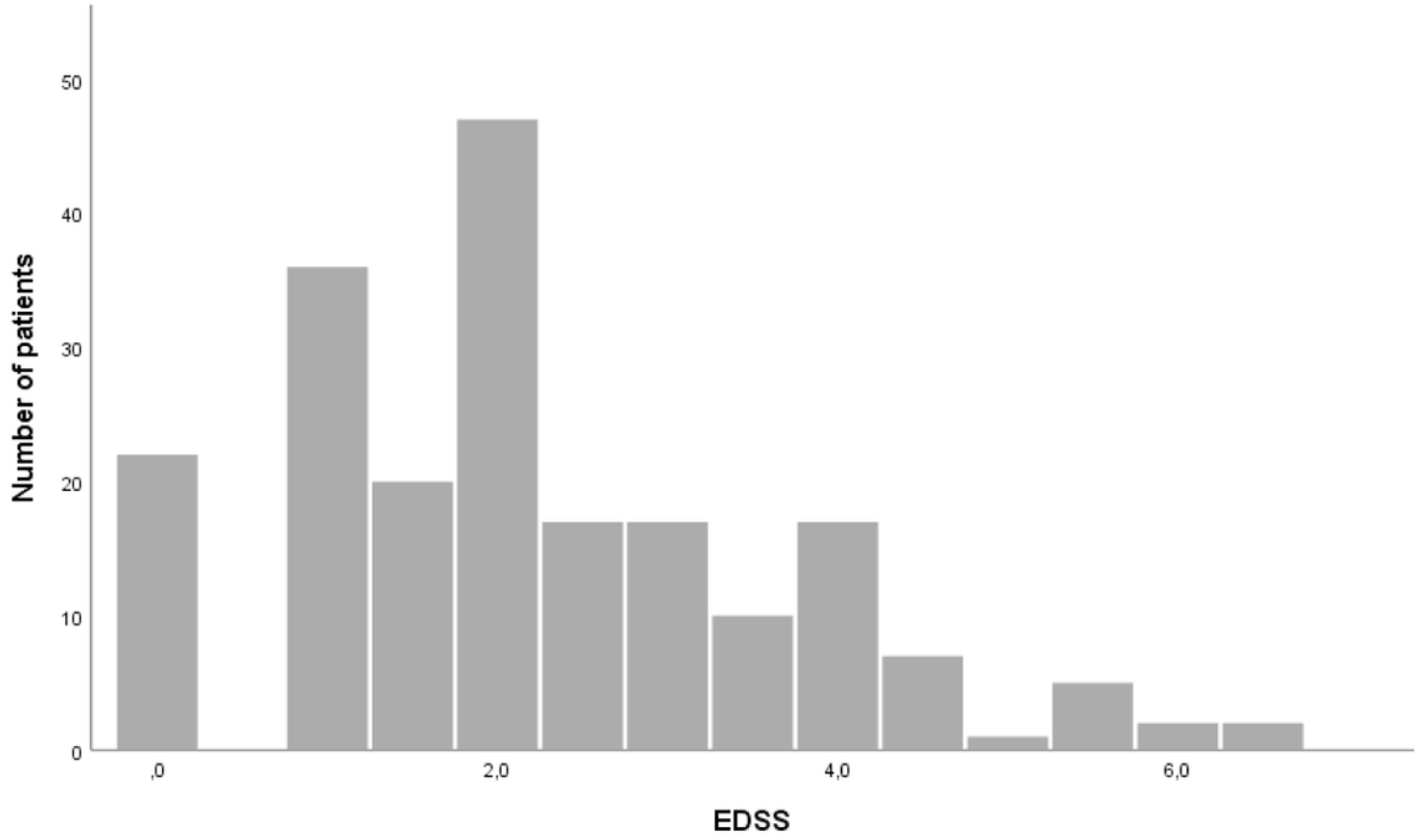

Figure 2

EDSS score at treatment start with NTZ

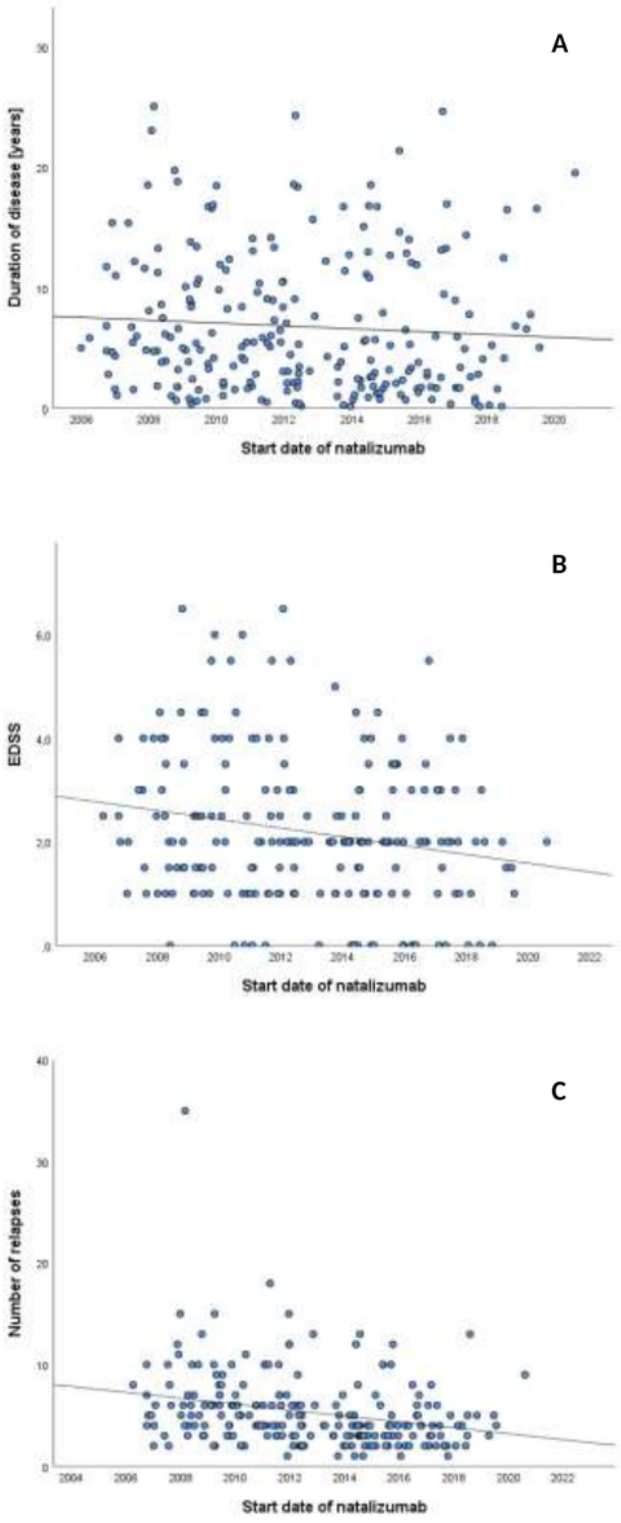

Figure 3

Scatter blots representing regression models of disease duration, EDSS score and number of relapses before start of NTZ over time Legend: Linear regression models of disease duration (A), EDSS score (B) and number of previous relapses from disease onset (C) at time of NTZ treatment start from 2006 to 2020. EDSS score at NTZ treatment start and number of relapses before NTZ significantly decreased over

time reflecting change of clinical practice. Significance (p) and Pearson correlation coefficient (r) of regression models: A: p=0.139, r=-0.71. B: p=0.002, r=-0,198. C: p<0.001, r=-0.263.

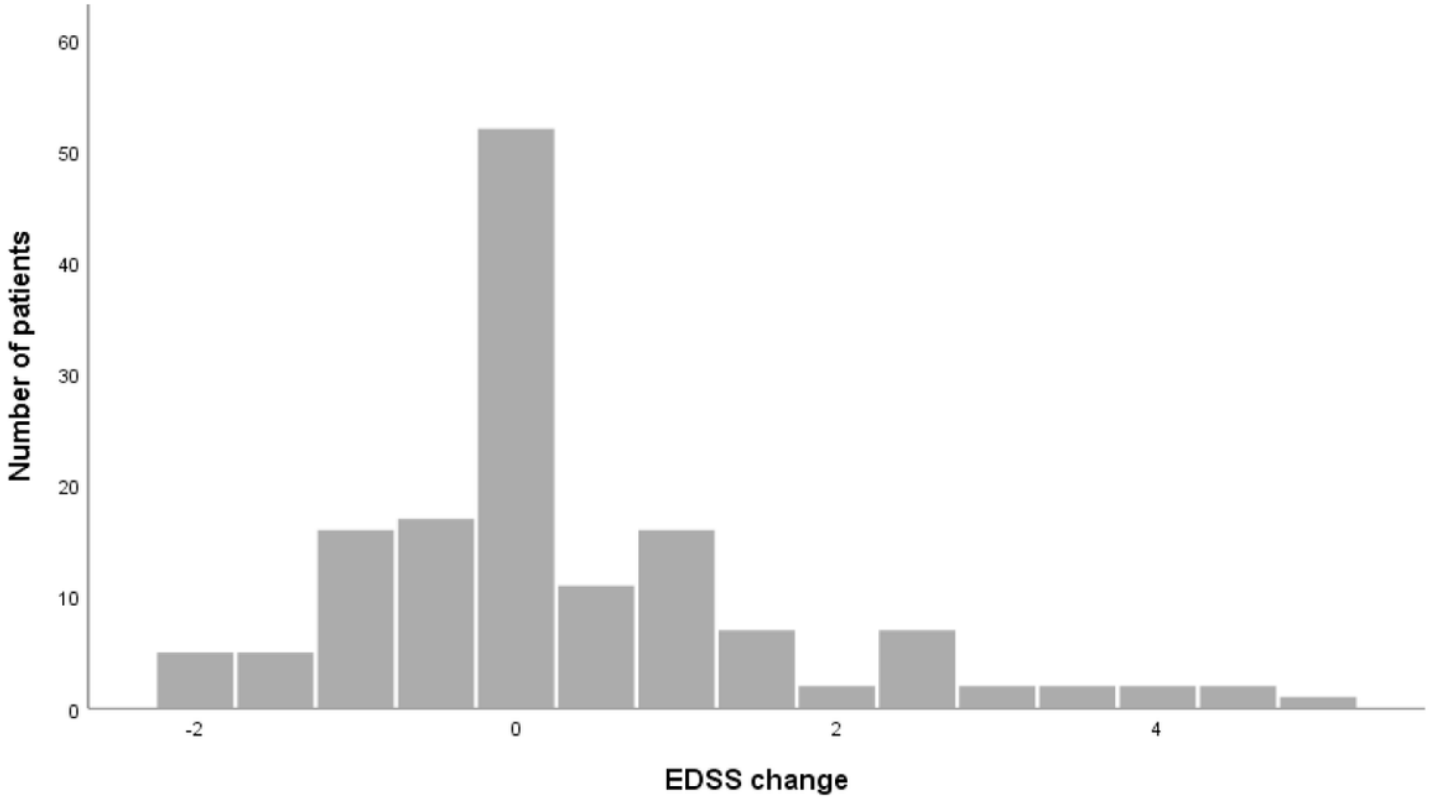

Figure 4

EDSS change on treatment with NTZ Legend: Change of EDSS from time of NTZ treatment start to last visit on treatment for patients with treatment duration ≥2 years.

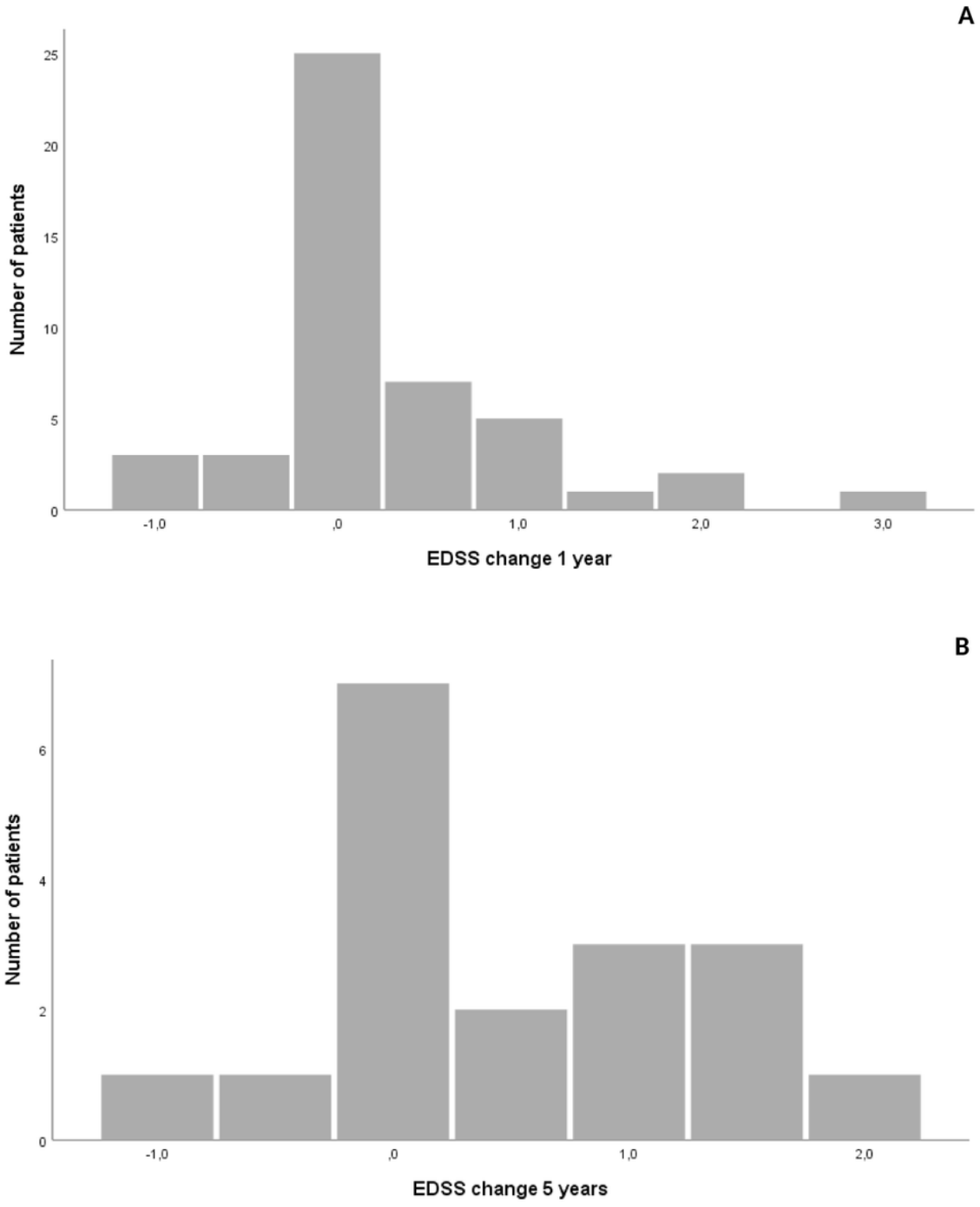

Figure 5

Change of EDSS score after NTZ discontinuation Legend: Change of EDSS score within one (A, 47 patients) and five years (B, 18 patients) from time of NTZ discontinuation. Only patients with a NTZ treatment duration ≥2 years were considered.