

The majority of natalizumab-treated MS patients have high natalizumab concentrations at time of re-dosing

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Abstract

Background: Natalizumab is efficacious in the treatment of relapsing-remitting multiple sclerosis. All patients receive the same treatment regimen of 300 mg every 4 weeks, despite differences in pharmacokinetics between individual patients.

Objective: To give neurologists insight into natalizumab concentrations at time of re-dosing, we investigated longitudinal natalizumab concentrations in 80 patients in relation to disease activity, with possible influencing factors.

Methods: In a prospective observational cohort study, natalizumab trough serum concentrations were measured in 80 patients. Data on demographics, duration of treatment, Expanded Disability Status Scale, clinical exacerbations, brain magnetic resonance imaging (MRI), and body weight were collected.

Results: We measured high ($\geq 10 \mu\text{g/mL}$) natalizumab trough concentrations in 94% of patients. Intra-individual concentrations were stable. The spread in concentrations was substantial and did not correlate with disease activity. We found a negative association between natalizumab concentration and body weight ($\beta = -0.30, p = 0.010$).

Interpretation: The majority of patients showed high natalizumab serum concentrations at time of re-dosing. Alternative treatment regimens could lead to more efficient use of natalizumab, but caution is warranted regarding the possibility of recurrence of disease activity. Prospective clinical trials are needed to establish the safety of extended dose intervals in natalizumab treatment.

Keywords: Multiple sclerosis, natalizumab, concentration

Introduction

Natalizumab (NTZ), targeting the α -4 integrin receptor, is an efficacious treatment for relapsing-remitting multiple sclerosis (RRMS).¹ In a phase-I trial, NTZ stayed detectable in the serum for 3–8 weeks after infusion with dosing of 1–3 mg/kg.² Based on the different therapeutic dosages of 3–6 mg/kg in phase-II trials, a fixed dose of 300 mg once in 4 weeks was chosen for phase-III trials so the majority of patients (with weights ranging between 50 and 100 kg) would fall between a dose of 3 and 6 mg/kg.³ Nowadays, a dose of 300 mg every 4 weeks has been approved by the European Medicines Agency (EMA)/Food and Drug Administration (FDA) for the treatment of RRMS. In this treatment regimen, NTZ concentrations may stay detectable in serum up to 200 days after cessation of therapy.⁴

Serum NTZ concentration corresponds with the percentage of α -4 integrin receptor saturation.⁵ Desaturation of the α -4 integrin receptor occurs when the serum NTZ concentration falls under 1–2 $\mu\text{g/mL}$.⁵ Above this threshold of 2 $\mu\text{g/mL}$, NTZ receptor saturation will roughly fall between 70% and 100%.⁵ An adequate receptor saturation is estimated as $\geq 70\%$ –80% saturation, although prospective data confirming this assumption are lacking.^{6,7} Based on a model with results from a large phase-II trial, approximately 90% of patients showed NTZ trough concentrations largely exceeding 2.5 $\mu\text{g/mL}$. Levels exceeding 2.5 $\mu\text{g/mL}$ could indicate that the approved treatment regimen of NTZ for RRMS results in a relative over-treatment, that is, the patient receives more NTZ than necessary for optimal drug efficacy.^{3,8} Furthermore, it is suggested that higher NTZ receptor

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saturation could increase the risk of progressive multifocal leukoencephalopathy (PML), the feared complication of NTZ treatment.⁹ This unconfirmed hypothesis leads to clinicians extending dose intervals in NTZ treatment with the aim of reducing the PML risk by decreasing NTZ exposure.^{9–11}

The aim of our study was to measure NTZ serum trough concentrations and correlate concentrations with disease activity and possible influencing factors.

Methods

Patients

In 2006, we initiated a prospective observational cohort study to monitor different aspects of NTZ treatment at the MS Centre of the VU University Medical Centre in Amsterdam, The Netherlands. All patients (nearly 220) starting NTZ have been included in this observational cohort. Patients in this cohort are annually subjected to a brain magnetic resonance imaging (MRI) and clinical testing including the Expanded Disability Status Scale (EDSS). For this study, we included all patients of the cohort who are currently treated with NTZ. Because NTZ concentration can fluctuate in the first year, mainly because of transient NTZ antibodies, we excluded patients with a NTZ treatment duration of less than 12 months.¹² All the patients included in this study received a strict treatment regimen of 300 mg NTZ every 4 weeks.

Measurement of NTZ concentration

Of all participants, blood samples were routinely obtained every 3 months before NTZ infusion. Serum was subsequently stored at –80°C at the biobank of the VU Medical Centre. For this study, we cross-sectionally measured NTZ concentrations of selected samples, using a cross-linking assay using polyclonal rabbit anti-NTZ F(ab)2 fragments for capture and a mouse anti-IgG4 monoclonal antibody for detection. This method, performed at Sanquin Laboratory, has recently been described in more detail.¹³ The detection limit of the assay is approximately 0.01 µg/mL.¹³ To investigate the stability of trough concentrations, we tested a second sample, with an interval of 3–7 months. Receptor desaturation can occur with serum concentrations of ≤ 2 µg/mL, which is our appointed cut-off point for an inadequate concentration.⁵ Taking into account the differences of individual pharmacodynamics, we assumed that serum concentrations of 2–10 µg/mL result in adequate receptor saturation. Concentrations exceeding 10 µg/mL do not result in higher receptor saturation

and is therefore labeled as the cut-off for high-trough concentration.⁵

Data collection

Demographic data, number of NTZ infusions, annual relapse rate before NTZ treatment, number of gadolinium enhancing lesions on the baseline scan, EDSS at start of NTZ treatment, John Cunningham virus (JCV) status at time of the measured concentration, clinical exacerbations during NTZ treatment, and body weight were assessed. The body weight was measured within 3 months of the blood sampling date. A clinical exacerbation was defined as new neurological symptoms lasting for more than 24 h and accompanied by new neurological signs found by a neurologist at the examination. All patients were subjected to a yearly MRI scan of the brain, including three-dimensional (3D) fluid-attenuated inversion recovery, axial proton density/T2-weighted sequences, and gadolinium-enhanced T1-weighted sequences. Those patients at higher risk for PML (JCV positive and >12 months on NTZ) were subjected to three monthly scans (without gadolinium except for the annual MRI scan) as is current recommended protocol.^{14,15} All MRI scans were evaluated by an experienced neuro-radiologist. The 2013 criteria of Lublin et al.¹⁶ were used when referred to “active MS.” According to these criteria, when referring to active MS, the patient experiences clinical relapses and/or occurrence of contrast-enhancing T1 or new/enlarging T2 lesions on brain MRI. In this study, we assessed MS activity starting 12 months after the start of NTZ treatment. If the patient experienced any clinical and/or radiological disease activity in the follow-up period, they were classified as “active MS.”

The local institutional review board approved the observational study and written informed consent was obtained from all participants.

Statistics

Continuous variables are expressed as mean and standard deviation if normally distributed or as median and interquartile range if not normally distributed. NTZ concentrations were normally distributed. For associating NTZ concentrations with different variables, we used the mean of the intra-individual longitudinal NTZ concentrations, except for the association with the number of NTZ infusions, in which we used the first measured NTZ concentration. For calculating the influence of different variables (body weight, NTZ infusions, age, and gender) on NTZ concentration, we used a linear regression model. For

Table 1. Demographic characteristics.

	Total, n=80
Age, mean (SD)	40.6 (10.1)
Gender, female, n (%)	55 (68.6)
Number of NTZ infusions, mean (SD) ^a	64.7 (32.2)
JCV positive, n (%)	29 (36.3)
EDSS at baseline NTZ, median (IQR)	3.3 (2.5)
ARR before NTZ start, mean (SD)	1.4 (0.9)
Number of gadolinium enhancing lesions at baseline, median (IQR)	1.5 (4)

SD: standard deviation; NTZ: natalizumab; EDSS: Expanded Disability Status Scale; IQR: interquartile range; ARR: annual relapse rate; JCV: John Cunningham virus.
^aNumber of NTZ infusions at the time of the first measured concentration.

calculating the influence of NTZ concentration on disease activity, we used a logistic regression model. Additional adjustments were made for confounding factors such as body weight, NTZ infusions, age, and gender.

All reported *p*-values are based on statistic tests, with a significance level set at <0.05. The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Approximately 220 patients have started NTZ treatment at the VU Medical Centre. At time of the start of this study, 101 patients were treated with NTZ. The main reason for discontinuing NTZ was the risk of PML, and other reasons were pregnancy-related reasons, allergic reactions, and progression to the secondary progressive phase. Of the 101 currently treated patients, 21 were excluded because of the treatment duration of less than 12 months. In the remaining 80 patients, 155 blood samples were tested for NTZ trough concentrations. Age of the patients ranged from 20 to 60 years. Patient characteristics are described in Table 1.

NTZ serum trough concentrations ranged from 0.1 to 80.0 µg/mL with a mean of 26.1 ± 14.1 µg/mL. In all, 1 patient (1.3%) had an inadequate concentration (<2 µg/mL), 4 patients (5%) had an adequate concentration (2–10 µg/mL), and 75 patients (93.8%) had a high concentration (≥ 10 µg/mL) at the time of redosing. The patient showing the lowest concentration (0.1 µg/mL at two measurements) appeared to have persistent high (>9000AE/mL) NTZ antibodies.

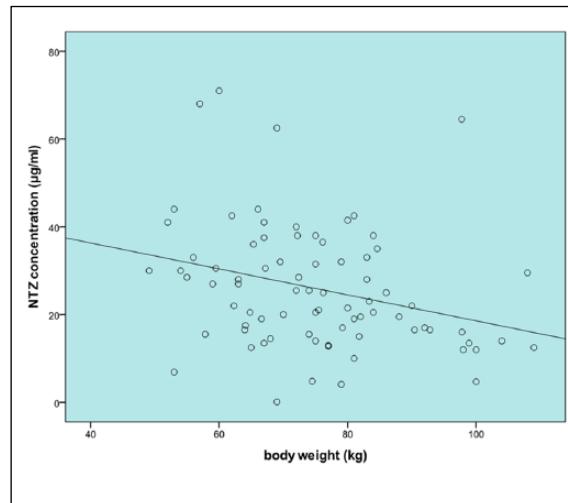


Figure 1. Body weight and NTZ trough concentration plot. An inverse association is found ($\beta = -0.30$, 95% CI = -0.52 to -0.07 ; $p = 0.010$; $r^2 = 0.084$).

Of 75 patients (93.8%), we measured a follow-up trough concentration. The mean concentration of all the cross-sectional samples did not differ (both 26.1 µg/mL) at the two different time points, that is, at group level, no rise or fall in concentration was observed. Longitudinal concentrations per patient fluctuated with a median difference of 3.0 µg/mL. In nine patients, the two samples differed more than 10 µg/mL; all these patients showed very high concentrations above 30 µg/mL.

The body weight of the patients ranged from 49.1 to 109.0 kg with a mean weight of 75.1 ± 13.9 kg. An inverse association was found between body weight and NTZ concentration (see Figure 1, $\beta = -0.30$, 95% confidence interval (CI) = -0.52 to -0.07 ; $p = 0.010$; $r^2 = 0.084$). We corrected body weight for potential confounders, but none of these variables appeared to be relevant. Patients weighing up to 75 kg showed a mean concentration of 29.2 ± 15.6 µg/mL, whereas the mean concentration of patients weighing 75 kg or more was 22.7 ± 11.9 µg/mL ($\beta = -6.6$, 95% CI = -12.8 to -0.34 ; $p = 0.039$).

There was no association found between the number of NTZ infusions and trough NTZ serum concentrations, with a substantial spread in concentrations regardless the duration of treatment (see Figure 2, $\beta = 0.022$, 95% CI = -0.092 to 0.113 ; $p = 0.84$).

The NTZ trough concentration was comparable between males and females, with a mean concentration of 25.0 ± 13.4 µg/mL and 26.9 ± 14.1 µg/mL, respectively ($\beta = -1.46$, 95% CI = -8.27 to 5.35 ;

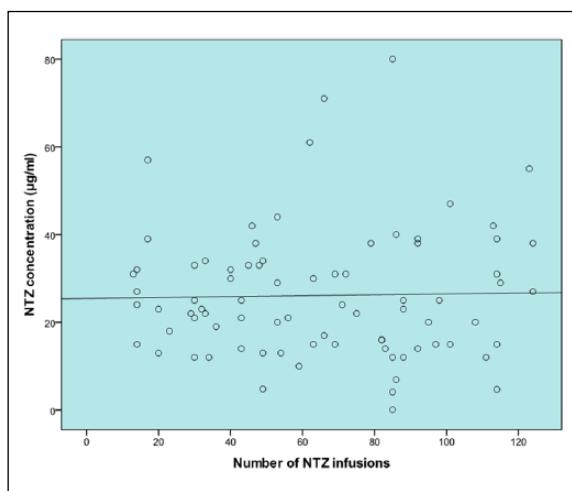


Figure 2. Number of NTZ infusions (duration of treatment) and NTZ trough concentration plot. NTZ concentration of the first measured sample is displayed with associated number of NTZ infusions. No association is found ($\beta=0.022$, 95% CI = −0.092 to 0.113; $p=0.84$). We do not see a rise in concentrations in long-term (5–10 years) NTZ users.

$p=0.67$). The concentration was not significantly associated with age ($\beta=-0.011$, 95% CI = −0.37 to 0.30; $p=0.94$).

Mean duration of NTZ treatment was 5.0 ± 2.5 years. A total of 15 patients (17.7%) had active disease under NTZ treatment (11 patients with new T2 lesions, 5 patients with a clinical exacerbation). Patients who had active disease under treatment received a median treatment duration of 5.1 years, and patients with non-active disease received a median treatment duration of 4.9 years. Mean concentrations were similar between patients with active and non-active disease with a mean concentration of 26.4 and 24.7 µg/mL, respectively (see Figure 3). When adjusting for body weight, concentrations were not statistically different for the active disease group versus the non-active disease group (odds ratio (OR)=0.98, 95% CI=0.94 to 1.03; $p=0.41$).

Discussion

NTZ is proven to be efficacious in the treatment of RRMS in a dosing schedule of 300 mg every 4 weeks. Despite large variations in patient pharmacokinetics, all NTZ-treated patients receive the same treatment regimen, where a personalized approach to the treatment schedule might be more appropriate.¹ Some neurologists are exploring extended dose intervals in order to reduce the risk of PML, although it is not confirmed that higher NTZ concentrations increase

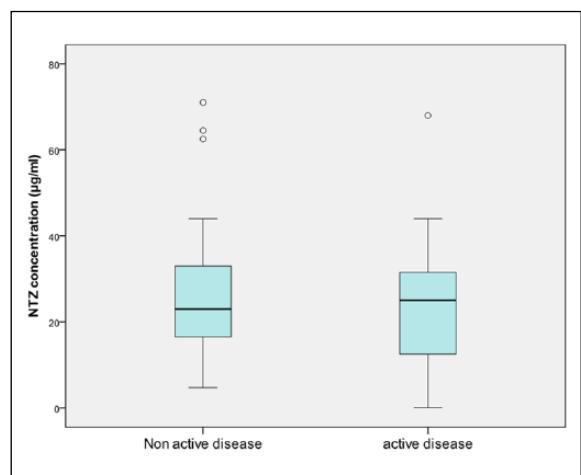


Figure 3. Active disease ($n=15$) versus non-active disease ($n=65$) (according to the 2013 Lublin criteria) and NTZ trough concentrations (OR=0.98; 95% CI=0.94 to 1.03; $p=0.41$).

the risk of PML.^{9,11} Obviously, modified treatment schedules should not interfere with drug efficacy. Our study addresses two important questions: (1) What is the proportion of high NTZ trough concentration in long-term-treated MS patients? and (2) Can we explain individual differences of NTZ concentrations?

In our study of 80 patients, 99% of patients showed adequate- to high-trough NTZ concentrations (≥ 2 µg/mL), with 94% having high (≥ 10 µg/mL) NTZ concentrations. The mean trough NTZ serum concentration in our cohort was above 20 µg/mL which is in agreement with recently presented data.¹⁷ The mean concentration was not lower in patients with active versus non-active disease, which suggests that high concentrations do not result in an increase in treatment efficacy in comparison with lower but still adequate concentrations. Considering this and the large proportion of high NTZ concentrations, NTZ could perhaps be administered less frequently (or with a lower dose) to reach NTZ concentrations that are lower but still cause adequate receptor saturation and consequently, optimal drug efficacy.⁵ Caution is advised though, because of a large spread in concentrations and the well-established rebound effect which occurs after cessation of NTZ treatment.¹⁸

In the RESTORE trial, 19% of patients ($n=23$) stopping NTZ, of whom the majority switched to another therapy, experienced a relapse within 28 weeks.¹⁹ The rebound effect showed an increase over time, although 8% of relapses occurred within 4–8 weeks of NTZ withdrawal. A large retrospective study, however,

showed no increase in disease activity with extending intervals up to 8 weeks and 5 days.⁹ In our study, 6.25% of patients showed either inadequate or adequate NTZ concentrations at time of re-dosing. For this group, extending intervals might result in rapidly falling concentrations under the therapeutic level and consequently an early rebound effect.

The large spread in NTZ trough concentrations could be explained by the variation in patient pharmacokinetics and characteristics. We associated age, sex, body weight, duration of treatment, and disease activity with NTZ concentrations. The only factor associated with NTZ concentration was body weight. This is in agreement with earlier reports, where some studies suggested a dose modification based on patients' body weight.^{20,21} Our results indeed confirmed an inverse association but this correlation was weak, only accounting for less than 10% of the variability in NTZ concentration. Therefore, body weight is an unreliable predictor for NTZ concentration.

NTZ can be found in serum up to 6 months after cessation of therapy.⁴ It has been suggested that NTZ concentrations increase over time in individual patients.²⁰ In our study, in 75 patients with a second measurement of NTZ trough concentration, the intra-individual concentrations were stable. Although we do not present long-term longitudinal follow-up trough NTZ serum concentrations, we did not find a correlation between duration of treatment and NTZ concentration. These data highlight that we should not expect very high concentrations in long-term NTZ-treated patients (> 5 years).

A limitation of this study is that measurements of saturation of the α -4 integrin receptor are lacking. Previous studies show that NTZ concentration is correlated with the α -4 integrin receptor saturation; however, above a certain concentration threshold, the receptor will be fully saturated (75%–100%). Based on available literature, we estimated this threshold to be 10 μ g/mL NTZ serum concentration, but this cut-off point needs to be confirmed in larger trials. Furthermore, if measuring NTZ levels, it is of importance to realize that the drug is a wild-type IgG4 antibody that becomes monovalent in vivo via "Fab arm exchange." This will affect concentration measurements to various degrees.^{13,22} Comparative studies between assays will be necessary to eliminate potential discrepancies between studies.

In conclusion, the large majority (94%) of NTZ patients have high NTZ trough concentrations which could be an indication that most patients receive a

"relative over-treatment." Extended dose intervals could help reduce costs of medication and increase quality of life for the patient with fewer hospital visits, but further studies are needed to establish the safety of alternative treatment regimens. We are now conducting a prospective clinical trial with concentration-based extended dose intervals in completely stable NTZ-treated patients, to assess whether concentration-based extended treatment regimens do not result in recurrence of disease activity. Results of such trials will hopefully give a decisive answer to the question whether extending dose intervals in NTZ treatment is feasible without losing drug efficacy.

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Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Z.L.E.K., C.E.L., B.I.W., and A.V. have nothing to report; M.P.W. has received speaking fees from Janssen-Cilag, Gayer-Schering, and Biogen Idec; T.R. has received speaking fees from Pfizer and AbbVie; J.K. has received speaker and consulting fees from Merck Serono, Biogen Idec, TEVA, Genzyme, Roche, and Novartis. VUmc MS Centre Amsterdam has received financial support for research activities from Bayer Schering Pharma, Biogen Idec, Roche, GlaxoSmithKline, Merck Serono, Novartis, and TEVA.

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