CHAPTER 3

THE MAJORITY OF NATALIZUMAB-TREATED MS PATIENTS HAVE HIGH NATALIZUMAB CONCENTRATIONS AT TIME OF RE-DOSING

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*Multiple Sclerosis Journal* 2018;24(6), 805-810
ABSTRACT

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

Objective
To give neurologists insight in 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 MRI and body weight were collected.

Results
We measured high (≥10μg/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 (β=-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.
INTRODUCTION

Natalizumab (NTZ), targeting the $\alpha_4$-integrin receptor, is an efficacious treatment for relapsing-remitting multiple sclerosis (RRMS). In a phase I trial, NTZ stayed detectable in the serum for 3-8 weeks after infusion with dosing of 1-3 mg/kg. Based on the different therapeutic dosages of 3-6 mg/kg in phase II trials, a fixed dose of 300mg once in four weeks was chosen for phase III trials so the majority of patients (with weights ranging between 50 and 100kg) would fall between a dose of 3 to 6 mg/kg. Nowadays, a dose of 300mg every four weeks has been approved by the EMA/FDA for the treatment of RRMS. In this treatment regimen, NTZ concentrations may stay detectable in serum in up to 200 days after cessation of therapy.

Serum NTZ concentration corresponds with the percentage of $\alpha_4$-integrin receptor saturation. Desaturation of the $\alpha_4$-integrin receptor occurs when the serum NTZ concentration falls under 1-2 μg/ml. Above this threshold of 2 μg/ml, NTZ receptor saturation will roughly fall between 70-100%. An adequate receptor saturation is estimated as ≥70-80% saturation, although prospective data confirming this assumption are lacking. Based on a model with results from a large phase II trial, approximately 90% of patients showed NTZ trough concentrations largely exceeding 2.5 μg/ml. Levels exceeding 2.5 μg/ml could indicate that the approved treatment regimen of NTZ for RRMS results in a relative over-treatment; i.e. the patient receives more NTZ than necessary for optimal drug efficacy. Furthermore, it is suggested that higher NTZ receptor saturation could increase the risk of progressive multifocal encephalopathy (PML), the feared complication of NTZ treatment. This unconfirmed hypothesis leads to clinicians extending dose intervals in NTZ treatment with the aim to reduce the PML risk by decreasing NTZ exposure.

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 MRI and clinical testing including the Expanded Disability Status Scale (EDSS). For this present 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 less than 12 months. All the patients included in this study received a strict treatment regimen of 300mg natalizumab every four weeks.

**Measurement of NTZ concentration**
Of all participants, blood samples were routinely obtained every three 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. The detection limit of the assay is approximately 0.01 μg/ml. To investigate the stability of trough concentrations we tested a second sample, with an interval of 3 to 7 months. Receptor desaturation can occur with serum concentrations of ≤2 μg/ml, which is our appointed cut-off point for an inadequate concentration. Taking into account the differences of individual pharmacodynamics, we assumed that serum concentrations of 2 to 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.

**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, JCV status at time of the measured concentration, clinical exacerbations during NTZ treatment and body weight were assessed. The body weight was measured within three months of the blood sampling date. A clinical exacerbation was defined as new neurological symptoms lasting for more than 24 hours 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 3D fluid-attenuated inversion recovery, axial PD/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 3-monthly scans (without gadolinium except for the annual MRI scan) as is current recommended protocol. All MRI-scans were evaluated by an experienced neuro-radiologist. The 2013 criteria of Lublin et al 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 or enlarging T2 lesions on brain MRI. In the present study we assessed MS activity starting 12 months after 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 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, 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 a 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 one.

Table 1. Demographic characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total, n=80</th>
</tr>
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<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>40.6 (10.1)</td>
</tr>
<tr>
<td>Gender, female n (%)</td>
<td>55 (68.6)</td>
</tr>
<tr>
<td>Number of NTZ infusions, mean (SD)*</td>
<td>64.7 (32.2)</td>
</tr>
<tr>
<td>JCV positive, n (%)</td>
<td>29 (36.3)</td>
</tr>
<tr>
<td>EDSS at baseline NTZ, median (IQR)</td>
<td>3.3 (2.5)</td>
</tr>
<tr>
<td>ARR before NTZ start, mean (SD)</td>
<td>1.4 (0.9)</td>
</tr>
<tr>
<td>Number of gadolinium enhancing lesions at baseline, median (IQR)</td>
<td>1.5 (4)</td>
</tr>
</tbody>
</table>

SD, standard deviation; NTZ, natalizumab; EDSS, Expanded Disability Status Scale; IQR, interquartile range; ARR, annual relapse rate; JCV, John Cunningham virus.

*Number of NTZ infusions at time of the first measured concentration

NTZ serum trough concentrations ranged from 0.1 μg/ml to 80.0 μg/ml with a mean of 26.1 ±14.1 μg/ml. One patient (1.3%) had an inadequate concentration (< 2 μg/ml), 4 patients (5%) had an adequate concentration (2 to 10 μg/ml) and 75 patients (93.8%) had a high concentration (≥10 μg/ml) at time of re-dosing. The patient showing the lowest concentration (0.1 μg/ml at two measurements) appeared to have persistent high (>9500 AE/ml) NTZ antibodies.

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, i.e. 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 9 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, β = -0.30, 95% C.I. -0.52 to -0.07; p = 0.010; r² = 0.084). We corrected body weight for potential confounders, but none of these variables appeared to be relevant. Patients weighing up to 75kg showed a mean concentration of 29.2 ±15.6 μg/ml, whereas the mean concentration of patients weighing 75kg or more was 22.7 ±11.9 μg/ml (β = -6.6, 95% C.I. -12.8 to -0.34; p = 0.039).

**Figure 1.** Body weight and NTZ trough concentration plot.

An inverse association is found (β = -0.30, 95% C.I. -0.52 to -0.07; p = 0.010; r² = 0.084).

There was no association found between the number of NTZ infusions and trough NTZ serum concentrations, with a substantial spread in concentrations regardless duration of treatment (see figure 2, β = 0.022, 95% C.I. -0.092 to 0.113; p = 0.84).
Figure 2. Number of NTZ infusions (duration of treatment) and NTZ trough concentration plot.

The NTZ trough concentration was comparable between males and females, with a mean concentration of 25.0 ±13.4 and 26.9 ±14.1 μg/ml respectively ($\beta = -1.46$, 95% C.I. -8.27 to 5.35, $p = 0.67$). The concentration was not significantly associated with age ($\beta = -0.011$, 95% C.I. -0.37 to 0.30; $p = 0.94$).

Mean duration of NTZ treatment was 5.0 ±2.5 years. 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, 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 μg/ml 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 (OR 0.98, 95% C.I. 0.94 to 1.03; \(p = 0.41\)).

**Figure 3.** Active disease versus non active disease

\[\begin{array}{c}
\text{NTZ concentration (\(\mu\)g/ml)}
\end{array}\]

![Box plot showing NTZ trough concentrations for active and non-active disease groups.](image)

*Active disease (n = 15) versus non active disease (n = 65) (according to the 2013 Lublin criteria) and NTZ trough concentrations (OR 0.98, 95% C.I. 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 300mg every four weeks. Despite large variations in patient pharmacokinetics, all natalizumab treated patients receive the same treatment regimen, where a personalized approach to the treatment schedule might be more appropriate.\(^3\) Some neurologists are exploring extended dose intervals in order of reducing the risk of PML, although it is not confirmed that higher NTZ concentrations increase 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. 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 of treatment efficacy in comparison to 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. Caution is advised though, because of a large spread in concentrations and the well-established rebound effect which occurs after cessation of NTZ treatment.

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. 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 five days. 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. 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 six months after cessation of therapy. It has been suggested that NTZ concentrations increase over time in individual patients. 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. This data highlights that we should not expect very high concentrations in long-term NTZ treated patients (> 5 years).

A limitation of the present study is that measurements of saturation of the α4-integrin receptor are lacking. Previous studies show that NTZ concentration is correlated with the α4-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 μg/ml NTZ serum concentration, but this cut-off point needs to be confirmed in larger trials. Furthermore, if measuring natalizumab 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 if extending dose intervals in natalizumab treatment is feasible without losing drug efficacy.

**ACKNOWLEDGEMENTS**

The authors wish to thank all patients included in the study for agreeing to the use of their data for research and education purposes. We thank Ms. A. Kalei for her help in the logistics regarding the measurement of sample concentrations. We also thank Ms. L. Balk for her advice regarding the statistical analyses.
Chapter 3

FUNDING

We acknowledge the support from the Brain Foundation Netherlands HA2015.01.05.
REFERENCES


