CHAPTER 9

NATALIZUMAB ASSOCIATED PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY IS NOT PRECEDED BY ELEVATED DRUG CONCENTRATIONS

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ABSTRACT

Background
In recent years, a small but increasing number of neurologists choose to extend dose intervals of natalizumab with the aim of reducing the risk of progressive multifocal leukoencephalopathy (PML). This idea is based on the hypothesis that high drug concentrations increase the risk of PML.

Objective
We invested the relation between longitudinal natalizumab concentrations in patients who developed PML and patients who did not develop PML.

Methods
In a prospective observational cohort study of 219 patients with relapsing-remitting multiple sclerosis treated with natalizumab, serum samples were taken every 12 weeks prior to natalizumab infusion. In this cohort, five patients developed PML and were matched with 10 patients from the cohort who did not develop PML. Natalizumab concentrations were measured in available samples and the longitudinal results were compared between the two patient groups.

Results
Mean natalizumab concentrations in the five patients developing PML was 18.9 µg/ml (SD ±13.4) versus 23.8 µg/ml (SD ±11.5) of the control patients. Furthermore, we did not observe a clear rise in concentration levels in patients subsequently developing PML.

Conclusion
Our results provide preliminary evidence that contradict the hypothesis that exposure to elevated concentrations of natalizumab is a relevant risk factor of developing PML.
INTRODUCTION

Natalizumab (NTZ) (Tysabri® Biogen Idec), a monoclonal antibody directed to \( \alpha_4 \) integrin, is an effective treatment in relapsing-remitting multiple sclerosis (RRMS)\(^1\). Progressive multifocal leukoencephalopathy (PML), a potentially fatal JC virus related opportunistic infection of the central nervous system (CNS), is a well-known adverse event of NTZ treatment. Consequently, most neurologists hold certain restraints in prescribing NTZ. Over the past decade, risk factors for developing PML have been described and risk analyses have been developed to guide neurologists and patients in their decision making in treating and monitoring JCV positive patients with NTZ\(^2\)\(^-\)\(^5\).

The standard treatment regimen is an infusion with 300mg NTZ every four weeks. An 80% saturation of the \( \alpha_4 \) integrin receptor is believed to achieve an optimal immunosuppressive effect in the CNS for treating RRMS\(^6\). Concentrations of NTZ correlate with the receptor saturation where desaturation of the receptor, described as <50% saturation, occurs when the concentration falls under 1µg/ml\(^7\). After 12 months, the large majority of patients have levels of NTZ exceeding 10µg/ml at the time of the next infusion, which might result in receptor saturations reaching near 100%\(^7\)\(^,\)\(^8\).

In order to reduce the risk of PML, an increasing number of neurologists adjust the standard treatment regimen to extend the interval to a maximum of eight weeks\(^9\)\(^,\)\(^10\). The hypothesis is based on the assumption that PML susceptibility reflects the percentage of receptor saturation with a higher risk when saturation is near 100%\(^9\). However, controlled studies in support of this hypothesis have not been reported.

The aim of our study was to investigate whether the concentration of NTZ is associated with the risk of developing PML.

METHODS

Data were derived from our local prospective observational cohort study including 219 RRMS patients treated with NTZ at the MS Centre of the VU University Medical Centre in Amsterdam. Blood samples were routinely obtained every 12 weeks before the infusion of NTZ (at the lowest point of serum NTZ concentration) and stored at \(-80^\circ\)C until assayed at Landsteiner
Laboratory Sanquin Research, Amsterdam. The local institutional review board approved the study and patients gave written informed consent.

Since 2006, five patients of our NTZ cohort have developed NTZ-associated PML. Four of these patients have been described previously in a paper addressing drug levels during plasma exchange after the diagnosis of PML. We matched the 5 PML patients with 10 long-term NTZ treated MS patients with respect to age and gender (with at least 7 years of NTZ treatment), and longitudinally measured serum NTZ concentrations. Because of the possibility of fluctuating NTZ concentrations due to mainly transient NTZ antibodies, we tested samples beginning 12 months after starting treatment.

Serum NTZ concentrations were measured in Sanquin laboratory, 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 as described. The detection limit of the assay is approximately 0.01 μg/ml. Lab measurements were performed by lab personnel blinded to the clinical data, unaware of who developed PML during follow-up.

Descriptive statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS, Inc., Chicago, IL, USA). To compare mean drug concentrations in samples of patients who developed PML (pre-PML samples) with samples of NTZ users not developing PML, the independent sample t-test was used.

RESULTS

The patient characteristics of PML patients and controls are described in table 1.

Table 1. Patient characteristics

<table>
<thead>
<tr>
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<th>PML patients (N=5)</th>
<th>Controls (N=10)</th>
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<tbody>
<tr>
<td>Mean age at start NTZ (range)</td>
<td>39 (29-55)</td>
<td>40 (28-51)</td>
</tr>
<tr>
<td>Sex, female%</td>
<td>60%</td>
<td>60%</td>
</tr>
<tr>
<td>Mean number of infusions (range)</td>
<td>43 (14-76)</td>
<td>106 (83-124)</td>
</tr>
<tr>
<td>JCV +</td>
<td>100%</td>
<td>50%</td>
</tr>
</tbody>
</table>

Characteristics of PML patients and controls. JCV measured by STRATIFY-2(8). NTZ=natalizumab. JCV=John Cunningham virus.
Four of the PML patients were asymptomatic at the time of PML diagnosis. The diagnosis was made based on the MRI findings including the lesion evolution on MRI and in four patients the diagnosis was further confirmed by the detection of JCV DNA by quantitative polymerase chain reaction in the cerebrospinal fluid. In the other patient, MRI lesions and lesion evolution were very characteristic for PML and MRI follow-up demonstrated a classical PML-immune reconstitution inflammatory syndrome (IRIS) five weeks after plasma exchange, as previously described. Prior to the diagnosis of PML, the time of the last serum sample available ranged from 1 day to 6 weeks.

A median number of 5 pre-PML samples were tested (range 2 to 8) in the PML patients. In addition, we tested yearly concentrations of 10 controls who were treated with NTZ for a minimum of 7 years. A total number of 96 samples were included.

The concentrations of the individual PML patients and mean concentration of the controls are set out in figure 1. The mean serum concentration prior to the diagnosis of PML was 18.9 µg/ml (SD ±13.4). The mean concentration of all yearly concentrations of the controls is 23.8 µg/ml (SD ±11.5). Neither the patients who developed PML, nor the controls showed a relevant rise of serum concentrations over time (see figure 1).

Of the 10 controls, 5 patients were repeatedly JC negative. The other 5 controls have been JC positive measured by a second-generation JCV antibody enzyme-linked immunosorbent assay (STRATIFY-2 test).
Figure 1. Natalizumab serum concentration

Graph of concentration of PML patients (continuous lines) and concentration of 10 controls (dotted line). The dotted line represents the mean concentrations of the 10 controls measures every year with standard deviations. Every continuous line represents a single patient developing PML at the end of the line. The serum concentration of the PML patients did not fluctuate more than 11 µg/ml during treatment.

- Last sample 2 weeks prior to diagnosis of PML
- Last sample 3 days prior to diagnosis of PML
- Last sample 1 day prior to diagnosis of PML
- Last sample 4 days prior to diagnosis of PML
- Last sample 6 weeks prior to diagnosis of PML
DISCUSSION

In our small but well-monitored and extensively sampled cohort of 219 patients, five patients developed PML. We did not observe any convincing difference between natalizumab serum concentrations of these PML patients when compared with 10 control patients. The mean concentration of the 10 controls (23.8 µg/ml) was in the same range as the mean concentration measured before the diagnosis of PML (18.9 µg/ml), not even showing a trend towards higher drug levels in pre-PML samples. In addition, the PML patients did not show a rise in concentration before the diagnosis of PML, neither did concentrations fluctuate more than 11 µg/ml during treatment of NTZ. Although this cohort is too small to draw definite conclusions, our results do not support the hypothesis of high serum concentrations as a risk factor for developing PML.

Furthermore, it has been suggested that after longer periods of NTZ treatment, concentrations can rise to considerable high levels\(^9\).\(^{14}\). A rise of concentration and concomitant receptor saturation over time, might explain the increased risk of PML after 24 months of treatment with NTZ. In our PML patients as well as the 10 controls we did not find a clear rise in NTZ serum concentration during long term follow-up.

The large retrospective study of Zhovtis et al., found no increase in disease activity with extending intervals in NTZ treatment, but prospective studies are needed to confirm that extending dose intervals does not affect efficacy\(^9\). Regarding the substantial inter-individual differences in NTZ concentration due to factors like weight and metabolism, it may be safer to extend NTZ intervals guided by serum NTZ concentrations. Rebound disease activity is a well-described phenomenon after cessation of natalizumab and obviously should be avoided\(^{15}\). Lessening infusion frequency while maintaining drug efficacy has advantages for the patient (decreasing hospital visits) and will have huge financial benefits. Besides these clear advantages, neurologists are extending dose intervals of natalizumab with the primary aim of reducing the risk of PML by lowering the NTZ exposure per patient. The study of Zhovtis et al. reported 4 PML cases in the non-extended dosing group and no PML cases in the extended group, although no statistical significance was found between extended and non-extended intervals. With our small cohort in mind and insufficient data on this subject so far, neurologists should be careful in extending dose intervals and not overstate a possible decrease of
the risk in developing PML. Obviously, if neurologists choose to extend dosing intervals of NTZ in JC positive patients, these patients should still receive stringent PML monitoring according to current recommendations\textsuperscript{2}.

We conclude that our results contradict the hypothesis of long-term exposure to high NTZ concentrations as a risk factor in developing PML. Furthermore, we do not observe a rise of NTZ serum concentration in long-term follow-up NTZ patients.

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REFERENCES


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