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Highlights

- Natalizumab has shown to impact fatigue, depression and cognitive dysfunction in multiple sclerosis.
- About half of patients, however, describe a subjective loss of this symptomatic effect at the end of each administration cycle.
- We could not objectify this loss of symptomatic effect as measured by scales for mood, fatigue and cognition in these patients.
- We could not identify changes in serum IL-6, TNF-α or IFN-γ as a potential explanation for symptom recrudescence.
End of dose interval symptoms in patients treated with natalizumab: a role for serum cytokines?

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Abstract

Background: Many natalizumab treated patients experience end of dose interval (EDI) symptoms towards the end of the administration cycle. Natalizumab has previously shown to influence cytokine profiles in relapsing remitting MS patients. We hypothesize that EDI symptoms might be explained by variability in serum cytokine levels during natalizumab treatment.

Methods: 42 relapsing remitting MS patients were included. Participants were evaluated before natalizumab administration (day 0) and 7 days afterwards (day 7). At both time points fatigue, depressed mood and cognition were evaluated using the fatigue severity scale (FSS), the visual analogue scale for fatigue (VAS-F), the symbol digit modality test (SDMT) and the inventory for depressive symptomatology (IDS-SR). Serum samples were tested for concentrations of IL-6, IFN-γ and TNF-α at both timepoints. On day 7 an additional EDI questionnaire was completed. Data were analyzed with SPSS by means of non-parametric tests.

Results: EDI symptoms were reported by 59.5%. Although fatigue was most frequently reported, fatigue scales did not significantly change from day 0 to 7 in (fatigued) EDI patients. Mood and cognition significantly ameliorated in both EDI and non-EDI patients. Cytokines remained stable at day 0 vs 7 except for a significant increase in IFN-γ. On day 0, IFN-γ concentration was positively correlated with a depressed mood in the whole cohort, and with mood and fatigue in the EDI group. Depressed mood positively whilst cognition negatively correlated with IFN-γ concentration on day 0 in the EDI subgroup reporting fatigue. No significant correlations between IL-6 nor TNF-α and symptom scores could be found.

Conclusion: In our study EDI symptoms could not be objectified since EDI and non-EDI groups did not differ in terms of change in mood, cognition and fatigue between day 0 and 7, suggesting that symptom recrudescence could be a subjective experience. Although our results need to be interpreted cautiously, we found no clear correlation between studied serum cytokines concentrations and the occurrence of EDI symptoms.

Keywords

Natalizumab, extended interval dosing, Multiple Sclerosis, end of dose interval symptoms, serum cytokines

1. Introduction
Natalizumab is a humanized monoclonal antibody against α4-integrin located on the surface of mononuclear cells, thereby interfering with and reducing the migration of lymphocytes across the blood-brain barrier. It has proven to be an efficacious treatment for relapsing remitting multiple sclerosis (RRMS) in terms of reducing disability progression, relapse rate and new MRI lesions (1, 2). Moreover, natalizumab showed to have an impact on fatigue, depression and cognitive functions (3, 4), incapacitating problems in a majority of MS patients. Although natalizumab demonstrated to be a highly effective and relatively safe agent for patients diagnosed with RRMS, it is associated with the rare occurrence of progressive multifocal leukoencephalopathy (PML) caused by the reactivation of the JC virus (5, 6). In natalizumab-treated patients with treatment duration for more than 2 years, a positive JCV serology, history of other immunosuppressive agents and/or high antibody index, the incidence of PML reaches up to 1% annually (5). It is suspected that PML susceptibility results from the natalizumab induced blockade of lymphocyte trafficking into the CNS, resulting in an excessive local reduction of immunocompetent cells required for JCV surveillance (5, 6). It is proposed that the risk of PML in natalizumab-treated patients can be decreased by extended interval dosing (EID) leading to a decreased α4-integrin receptor saturation without influencing the agent’s clinical efficacy, but thereby enabling a certain degree of immunosurveillance in the CNS that might be required for the prevention of PML. This hypothesis is supported by the analysis from the TOUCH Prescribing Program and the Mitigation Strategy program (REMS) reporting that extended interval dosing dramatically reduces the risk of PML (7). Several studies – although their results were inconclusive and limited by the non-randomized designs, small sample sizes and variable definition of EID – indeed showed that an off-label use of a dosing interval of 5 to 8 weeks can be applied without affecting the clinical efficacy in terms of relapse rate, disability progression and MRI parameters (5, 6). However, many natalizumab-treated patients declare they need their next monthly infusion as a “boost”, experiencing a “wearing off” by the end of each administration cycle (so called end of dosing interval (EDI) symptoms). A study evaluating fatigue in MS patients treated with natalizumab was able to find an objective cyclic effect on symptom recrudescence in about half of patients treated with natalizumab (8). The occurrence of EDI symptoms might complicate extended interval dosing in an attempt to reduce PML risk. Most frequently reported EDI symptoms such as fatigue, weakness, walking impairment and cognitive difficulties severely interfere with quality of life making extended interval dosing possibly less attractive for those patients. However, the known mechanism of action and pharmacokinetics of natalizumab cannot adequately explain this phenomenon of EDI symptoms.

Peripheral cytokines have been evaluated as serum markers of fatigue in MS but only interleukin-6 (IL-6) has shown to correlate with fatigue scales (11). Apart from fatigue, serum IL-6 levels also seem to correlate with depression in MS (12) and higher IL-6 production by peripheral blood mononuclear cells correlate with poorer
performances in cognitive tasks in MS (13). Although less clear interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) have also been implicated in MS fatigue as witnessed by whole blood stimulation assays (14, 15). Depression during relapses correlated with increased TNF-α and IFN-γ mRNA in white blood cells (16) and serum TNF-α has shown to be increased in depressed MS patients (17). Natalizumab has also shown to influence serum cytokine profiles in MS patients (9, 10). However, so far only long term effects were studied and short-term cytokine kinetics during natalizumab treatment have never been investigated. Variability in the serum cytokine kinetics during natalizumab treatment may explain EDI symptoms in some patients. In this study we aimed to further explore the phenomenon of EDI symptoms and evaluate the short-term effects of natalizumab standard dose (300 mg IV/4weeks) on fatigue, cognition and mood in patients with or without EDI symptoms and their potential correlation with serum cytokines.

2. Materials and Methods

2.1 Patients

Relapsing remitting MS patients were recruited from the 4 hospitals in Ghent, Belgium (University Hospital Ghent, AZ Maria Middelares, AZ Sint-Lucas and AZ Jan Palfijn) from February 2017 until March 2018. All patients diagnosed with RRMS receiving monthly intravenous natalizumab who met inclusion criteria were invited to participate. Patients who experienced an infection or MS relapse two months prior to inclusion were excluded from participation. Forthy-three patients agreed to participate. All participants signed an informed consent before entering the study. Approval of the Ethics committee of the UZ Gent was obtained (reference number: 2017/0020) and approved by the local ethics committees of participating centers.

2.2 Assessments

2.2.1. Fatigue, mood and cognition

Participants were evaluated at two time points: on the day of infusion prior to natalizumab administration (day 0) and 7 days after receiving natalizumab (day 7). At both time points, participants were asked to rate their level of fatigue in the past 7 days using the visual analogue scale for fatigue (VAS-F) and the fatigue severity scale (FSS). In the VAS-F, subjects were asked to place an ‘X’ on a 10 cm-line representing their fatigue level yielding a score from 0 ‘not at all tired’ to 10 ‘extremely tired’. The FSS is a survey consisting of 9 statements concerning fatigue in which patients are asked to score their level of agreement from 1 ‘totally disagree’ to 7
‘completely agree’ with higher scores indicating more severe fatigue. Depressive symptoms in the past week were assessed at both time points using the inventory of depressive symptomatology (IDS-SR), a standard 30-question survey in which the score for each question varies from 0 to 3, with higher scores suggesting more severe depressive symptomatology. Cognitive function was evaluated at both time points using the same symbol digit modality test (SDMT) in which patients are given 90 seconds to link given geometric figures to specific numbers using a reference code. On day 7 an additional questionnaire on recurrence of symptoms at the end of the natalizumab interval was completed. Patients were asked whether or not they experienced certain specific re-occurring symptoms towards the end of the dosing cycle responsive to natalizumab re-infusion. If patients indicated they recognized this phenomenon, they were asked to write down the specific experienced symptoms. This EDI questionnaire was filled out after all previous tests were completed and patients were unaware of the intention to study EDI symptoms until after this questionnaire. Patients were then divided in two groups based on the occurrence of EDI symptoms (EDI group and non-EDI group).

2.2.2 Serum Cytokine analysis

Blood samples were taken on day 0 before natalizumab administration and 7 days afterwards (day 7). Blood was collected into serum tubes and transferred to the laboratory for storage within two hours. Serum samples were tested for concentrations of IL-6, IFN-γ, TNF-α. Ultrasensitive ELISA kits BMS213HS, BMS228HS and BMS223HS (Thermo Fisher) were used for quantitative detection of serum IL-6, IFN-γ and TNF-α respectively on both time points, following manufacturer’s instructions. The detection limit that was used for statistical analysis was determined as OD blanco + SD x2, which is < 0.14 pg/ml for IFN-γ, < 0.16 pg/ml for IL-6 and < 1.25 pg/ml for TNF-α). Serum concentrations below the proposed detection limit were replaced by the arbitrary value determined as (OD blanco + SDx2)/2.

2.2.3 Statistical Analyses

Patients were initially divided in two groups based on the occurrence of EDI symptoms. Patients who indicated on the EDI questionnaire that they experienced EDI symptoms were assigned to the EDI (n=25) group. Patients who did not report experiencing EDI symptoms were assigned to the non-EDI group (n=17). Since fatigue was the most reported EDI symptom (n=20), we performed an additional explorative analysis in those patients reporting fatigue as EDI symptom, the so called ‘fatigue-EDI subgroup’ (fEDI, n=20). Nonparametric tests were used for statistical analysis assuming a non-normally distributed population given the relatively small sample size. Comparisons among groups (EDI group versus non-EDI-group) in demographic characteristics, disease characteristics, test scores and cytokine concentrations were analyzed using the Fisher’s exact test to compare binomial
variables and the Mann-Whitney U test to compare continuous variables individually. Changes in test scores and cytokine concentrations from day 0 to day 7 were analyzed using the non-parametric paired Wilcoxon signed ranks test. Correlations between continuous variables reflecting fatigue, mood and cognition and cytokine concentrations, were assessed using Pearson’s rank correlation test. Significant outliers were identified using the Grubb’s test, only one data point in the IFN-γ analysis was identified as an outlier. All analyses were performed using SPSS statistical software version 26.0. P-values less than 0.05 were considered significant.

3. Results

3.1 Demographic data and disease characteristics

Forty-three RRMS patients receiving monthly natalizumab treatment recruited from 4 hospitals in Ghent, Belgium agreed to participate. One subject was withdrawn for being unable to comply with the study protocol leaving a total of 42 subjects for statistical analysis. Demographic and clinical characteristics are summarized in table 1.

Twenty-five patients (59.5%) reported EDI symptoms. Among those patients fatigue was most commonly reported (80%, fEDI group), followed by weakness (36%) and cognitive difficulties (28%) (table 2).

Comparing both groups, age, sex, disease duration, duration of natalizumab treatment, BMI, smoking status, alcohol consumption and EDSS score were similar (table 1).

3.2 Fatigue, mood and cognition

All participants completed surveys assessing mood, cognition and depressive symptomatology before natalizumab administration (day 0) using the VAS-F, FSS, SDMT and the IDS-SR. One week after natalizumab infusion, patients returned to the hospital to complete the same questionnaires (day 7). Figure 1, panel A summarizes all the results from these clinical measures.

3.2.1 Fatigue

On day 0, no significant differences were observed in mean (± standard deviation) VAS-F scores (EDI 5.71± 2.58, non-EDI 6.81±2.34, p= 0.086) or mean FSS scores (EDI 4.43±1.19, non-EDI 4.77±1.35, p=0.349) between both groups. On day 7 VAS-F scores were significantly higher in the non-EDI (6.57±2.19) as compared to the EDI
Mean VAS-F and FSS scores did not significantly change one week after natalizumab infusion in the whole cohort (VAS 6.15±2.52 to 5.72±2.26, p=0.116; FSS 4.56±1.25 to 4.59±1.28, p=0.844). Although fatigue was the most reported EDI symptom (80% of EDI-patients), nor FSS nor VAS-F scores significantly improved after natalizumab administration in the EDI group (FSS 4.43±1.19 to 4.36±1.21, p=0.823; VAS-F 5.71±2.58 to 5.14±2.15, p=0.238). We found no significant changes in FSS nor VAS-scores in the non-EDI group from day 0 to day 7 (FSS 4.77±1.35 to 4.93±1.35, p=0.602; VAS-F 6.81±2.34 to 6.57±2.20, p=0.115). Remarkably, even in the fEDI subgroup no significant improvement in FSS score nor VAS-F score was observed on day 7 compared to day 0 (4.52±1.25 to 4.43±1.19, p=0.679 and 5.99±2.53 to 5.35±1.95, p=0.248 respectively).

### 3.2.2 Cognition

On day 0 and day 7, mean SDMT scores did not significantly differ between the different subgroups. A significant improvement in mean SDMT score was seen in the whole cohort (40.83±12.03 to 45±13, p<0.001), the EDI group (41.84±11.83 to 47±13.11, p=0.002) as well as the non-EDI group (39.35±12.53 to 42.06±12.65, p=0.035) on day 7 compared to day 0. A similar improvement in SDMT score on day 7 versus day 0 was observed in the fEDI subgroup (41.4±12.83 to 47.25±14.19, p=0.003).

### 3.2.3 Depressive symptomatology

Mood parameters did not significantly differ between the different subgroups on day 0 and day 7 (day 0 p=0.617, day 7 p=0.228). Mean IDS-SR scores were significantly higher (indicating more severe depressive symptomatology) before natalizumab administration compared to 7 days after infusion in the whole cohort (20.143±11.39 to 16.57±11.08, p<0.001). A significant improvement in mood parameters as indicated by a significant reduction in mean IDS-SR was observed in both the EDI group as well as the non-EDI group 7 days after infusion (EDI 19.16±10.33 to 14.64±10.35, p=0.004; non-EDI 21.59±12.42 to 19.41±11.81, p=0.025). The same effect was observed when subgroup (fEDI group) analysis was performed, where IDS-SR scores turned out to be significantly lower on day 7 compared to day 0 in the fEDI group (19±10.85 to 15.95±10.81, p=0.026).

### 3.3 Cytokine analysis

Measured IFN-γ cytokine concentrations were low and most measured values were either undetectable or around the detection limit. An arbitrary value determined as (OD blanco + SDx2)/2 was used if measured concentration of IL-6 and IFN-γ were below the proposed detection limit. Since all but one value of measured TNF-α were below the detection limit, we could not perform this correction for this specific cytokine. IFN-γ values from one patient were left out the analysis since they were...
identified as significant outliers by the Grubb’s test. Panel B of figure one summarizes all the results from the cytokine analysis at day 0 and 7 for the different subgroups. Panel C illustrates the positive correlation found at day 0.

On day 0, we found a significant difference in mean IFN-γ concentration between both groups (EDI 0.12±0.10; non-EDI 0.23±0.18, p=0.026) and between the non-EDI and fEDI group (fEDI 0.11±0.08; non-EDI 0.23±0.18, p=0.018) whereas no significant differences in mean IL-6 nor TNF-α concentration were found at day 0 and at day 7 for the different subgroups. For the whole cohort, mean IFN-γ concentration was significantly higher on day 7 compared to day 0 (0.16 ±0.14 pg/ml to 0.34 ±0.62 pg/ml, p=0.035), whereas IL-6 and TNF-α levels remained stable on day 0 versus day 7 (IL-6 0.38±0.30 to 0.49±0.56, p=0.171; TNF-α 0.42±0.18 to 0.46±0.22, p=0.443). There was no significant increase in IFN-γ concentration at day 7 compared to day 0 in the EDI nor non-EDI group (EDI 0.12±0.10 to 0.30 ±0.65, p=0.177; non EDI 0.23 ±0.18 to 0.41 ±0.60, p=0.075). When the EDI and non-EDI group were compared, we observed no significant changes in IL-6 or TNF-α levels from day 0 to 7. In the fEDI group, no significant increase in IFN-γ concentration on day 7 compared to day 0 was observed (0.11±0.08 to 0.17±0.17, p=0.333).

The results of Pearson’s rank correlation test between IL-6, IFN-γ and TNF-α and VAS-F, FSS, SDMT and IDS-SR scores are summarized in panel C of figure 1. We found a significant positive correlation between IDS-SR score on day 0 and IFN-γ on day 0 (r=0.426, p=0.007) in the whole cohort. There were no significant correlations between cytokine concentrations and fatigue nor cognition on day 0 when the whole cohort was studied. We further found a significant positive correlation between IDS-SR score and FSS score and IFN-γ concentration on day 0 in the EDI-group (r=0.636, p=0.001 for mood and r=0.438, p=0.029 for fatigue). In the fEDI group, IFN-γ concentration on day 0 was positively correlated with IDS-SR scores (r=0.613, p=0.004) whilst it was inversely correlated with SDMT scores on day 0 (r=−0.579, P=0.007). We were not able to identify any significant correlations between IL-6 and TNF-α and fatigue, mood or cognition on day 0. On day 7 no significant correlations between test scores and cytokine levels were found.

4. Discussion

This study showed that a significant amount (59.5%) of natalizumab-treated RRMS patients experience EDI symptoms towards the end of the administration cycle. Among patients reporting EDI symptoms, fatigue was the most commonly reported symptom (80%) (table 1 and 2). We observed an improvement in scales for cognition and depressive symptomatology in the whole cohort 7 days after natalizumab infusion, ameliorations in EDI and non-EDI were comparable. At day 7 non-EDI
patients scored higher on the fatigue scales compared to EDI patients but not in comparison to fEDI patients. Amelioration in fatigue parameters between testing at day 0 and 7 could not be found, not even in the fEDI subgroup (panel A of figure 1). As such our findings could not objectify the EDI symptoms reported by patients. This is in contrast to earlier reported results published by Ratchford et al (8). According to their findings, EDI symptoms were experienced by a similar 57% of participants with fatigue, weakness, walking impairment and cognitive difficulties being most frequently reported. They did however report a significant improvement in fatigue and mood parameters one week after natalizumab infusion, an effect that was limited to the EDI group. In their study, mean FSS score and mean VAS-F were significantly lower 7 days after natalizumab administration in the EDI group (5.21 to 3.59, p<0.001 and 4.79 to 4.27, p=0.0012 respectively). However, the clinical relevance of a reduction in mean VAS-F score from 4.79 to 4.27 should be questioned. Another potential confounder is that participants were aware of the intention to study EDI symptoms, leading to a potential bias, which was not the case in our study.

We aimed to investigate short-term cytokine kinetics during natalizumab therapy as a potential mechanism behind EDI symptoms. Natalizumab has previously been shown to influence cytokine levels but effects were only studied in the long term. RRMS patients treated with natalizumab show an increased number of activated leukocytes producing pro-inflammatory cytokines in the peripheral blood (9, 18, 19). To our knowledge, we are the first to investigate the cytokine kinetics and their association with the occurrence of EDI symptoms throughout a standard 4-week natalizumab dosing cycle (panel B and C of figure 1 for a summary of findings). Our data show a significant increase in IFN-γ after natalizumab infusion in the whole cohort, whereas IL-6 and TNF-α remain stable. An increase in PBMC mRNA expression of IFN-γ after 12 months of natalizumab treatment was previously described by Khademi et al. (18). Ramos-Cejudo et al. (20) also demonstrated increased IFN-γ at the protein level as measured by flow cytometry after natalizumab administration with return to baseline before the second dose, in this paper short term kinetics where only studied in the first month of natalizumab treatment. A positive correlation between IFN-γ mRNA expression and depressive symptomatology has previously been reported in MS patients during acute relapses (16). We found a significant positive correlation between IDS-SR and IFN-γ concentration in the whole cohort and in the EDI group at day 0. IFN-γ was positively correlated with IDS-SR whilst it was negatively correlated with SDMT score on day 0 in the fEDI subgroup. However, the absence of correlation at day 7 make this a doubtful finding. IL-6, IFN-γ, and TNF-α have shown to be associated with MS fatigue (14, 15, 17). We found a positive correlation between FSS scores and IFN-γ in the EDI-group at day 0. Patanella et al. reported that high levels of IL-6 correlated with poorer performances in cognitive tasks in patients with RRMS (11). We found no correlation between IL-6 and cognitive function. In our study symptom recrudescence did not correlate with serum IL-6 or TNF-α levels. It is of great importance to notice however that our results need to be interpreted cautiously, since measured serum cytokine concentrations were low and most
measured values were around the detection limit. The used technique (ultrasensitive ELISA kits) might not have been sensitive enough to adequately pick up serum cytokine concentration making it hard to draw any firm conclusions.

Few papers have discussed the phenomenon of symptom recrudescence at the end of the dosing cycle. The mechanism that causes this fluctuation of symptoms is unknown. A first possible mechanism is that EDI symptoms occur as a consequence of low levels of CNS inflammation due to lower natalizumab levels at the end of the administration cycle thereby enabling lymphocytes to enter the CNS causing inflammation. However, the pharmacokinetics of natalizumab do not support this hypothesis since sufficient receptor saturation is maintained throughout a standard 4-week dosing cycle. This is supported by Kempen et al. who investigated whether the occurrence of EDI could be explained by a non-optimal pharmacokinetic/dynamic state (21). They included patients with RRMS on a standard 4-week dosing cycle and on extended interval dosing (5-8 weeks). The authors concluded that EDI symptoms were not associated with natalizumab concentration nor α-4 integrin receptor saturation. Furthermore, EDI symptoms were more commonly reported by patients on a standard dosing cycle compared to extended interval dosing. A second potential explanation is that natalizumab promotes or inhibits the release of soluble factors such as pro-inflammatory cytokines thereby causing the recurrence of symptoms. This is what we aimed to investigate in our study. Although our data do not support this hypothesis, we cannot reject it based on our results. The cytokine kinetics of only three cytokines were studied during one dosing interval whereas natalizumab has proven to influence multiple pro- and anti-inflammatory cytokines in the long term. As mentioned above, the sensitivity of the ultrasensitive ELISA kits that were used might have been insufficient since most of our measured values were either undetectable or around the detection limit. Future research with a broader range of cytokines and/or ex-vivo PBMC stimulation may further help to exclude a role for cytokines in EDI. A third hypothesis that should be considered is that experiencing EDI symptoms might be a subjective phenomenon, and that the improvement in fatigue, mood and cognition after natalizumab infusion might be due to a placebo effect. Patients might expect an improvement in symptomatology soon after natalizumab infusion, followed by subjective decline later on. Our results support this hypothesis since arguments for fatigue amelioration after treatment could not be found in the EDI group, not even in the fEDI subgroup and symptomatic effects in scales for mood and cognition were equally found in EDI and non-EDI patients. This hypothesis is also supported by the data presented by Katz et al. They reported that the majority of patients reporting EDI symptoms experienced those symptoms 4-9 days prior to each infusion, regardless of the dosing interval. Furthermore, they could not identify any significant difference in intensity, onset or duration of EDI symptoms in patients on a standard dosing schedule compared to those on a six-or eight-week dosing cycle (22).

Extended interval dosing is an off-label use of natalizumab which benefit-risk profile is
currently unknown. Our results encourage further investigation of EID in an attempt to reduce PML risk, since we could not objectify the occurrence of EDI symptoms.

5. Acknowledgements

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6. Disclosure

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Dekeyser Cathérine: has received travel compensation from Biogen;
De Pue Annelien: has received travel compensation from Biogen; Sieben Anne: nothing to disclose; Algoed Luc: has received travel compensation from Biogen; Vanhijfte Liesbeth: nothing to disclose; Sarah Gerlo: nothing to disclose;
Laureys Guy: has received travel compensations, consultancy and speakers fees from Biogen

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Table 1: Baseline characteristics (Abbreviations: End of dose interval (EDI); standard deviation (SD), Body mass index (BMI), Expanded disability status scale (EDSS))

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Whole cohort</th>
<th>EDI</th>
<th>non-EDI</th>
<th>P value</th>
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<tr>
<td><strong>Baseline &amp; Disease characteristics</strong></td>
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<tr>
<td>Number</td>
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<td>25</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>% female</td>
<td>57.1</td>
<td>48</td>
<td>70.6</td>
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<tr>
<td>Mean (SD) age, y</td>
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<td>42.08 (10.73)</td>
<td>40.35 (13.560)</td>
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<tr>
<td>Mean (SD) disease duration, y</td>
<td>9.72 (7.23)</td>
<td>8.48 (4.17)</td>
<td>11.5 (10.07)</td>
<td>0.474</td>
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<tr>
<td>Mean (SD) tysabri treatment duration, y</td>
<td>5.1 (2.7)</td>
<td>4.25 (1.96)</td>
<td>6.3 (3.18)</td>
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<tr>
<td>Mean (SD) BMI</td>
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<td>25.03 (4.26)</td>
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<td>Smoking, %</td>
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<td>Alcohol, %</td>
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<td>83.3</td>
<td>64.7</td>
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<td>EDSS mean (SD)</td>
<td>3.517 (2.11)</td>
<td>3.61 (2.01)</td>
<td>3.36 (2.36)</td>
<td>0.544</td>
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Table 2: Reported end of dose interval symptoms

<table>
<thead>
<tr>
<th>Symptom</th>
<th>% Reporting (n)</th>
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<tbody>
<tr>
<td>Fatigue</td>
<td>80 (20)</td>
</tr>
<tr>
<td>Weakness</td>
<td>36 (9)</td>
</tr>
<tr>
<td>Walking difficulties</td>
<td>4 (1)</td>
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<tr>
<td>Cognitive difficulties</td>
<td>28 (7)</td>
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<tr>
<td>Paresthesias</td>
<td>12 (3)</td>
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<tr>
<td>Numbness</td>
<td>12 (3)</td>
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<tr>
<td>Pain</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Blurry vision</td>
<td>12 (3)</td>
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<tr>
<td>Condition</td>
<td>Value</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Tinnitus</td>
<td>4 (1)</td>
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<tr>
<td>Balance disturbance</td>
<td>12 (3)</td>
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<tr>
<td>Sleep disturbance</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Agitation</td>
<td>16 (4)</td>
</tr>
<tr>
<td>Emotional</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Restless legs</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Decreased energy level</td>
<td>16 (4)</td>
</tr>
<tr>
<td>Headache</td>
<td>8 (2)</td>
</tr>
<tr>
<td>Itch</td>
<td>4 (1)</td>
</tr>
<tr>
<td>spasms</td>
<td>8 (2)</td>
</tr>
</tbody>
</table>
Figure 1: Panel A/ Clinical test scores (mean+SD) at day 0 and day 7 for the different subgroups of patients, statistical significance levels according to Mann-Whitney U test or Wilcoxon signed ranks test as appropriate. Panel B/ Serum cytokine levels (mean +SD) at day 0 and 7 for the different subgroups, statistical significance levels according to Mann-Whitney U test or Wilcoxon signed ranks test as appropriate. Panel C/ Statistically significant correlations found between clinical test scores and cytokine levels according to Pearson’s rank correlation test. (Legend: * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, day 0 data depicted in red, day 7 data in blue) (Abbreviations: All: complete patient cohort, EDI: end of dose interval patients, fEDI: fatigued EDI patients, nEDI: patients not experiencing EDI, FSS: Fatigue severity scale, IDS-SR: inventory of depressive symptomatology, SDMT: symbol digit modality test, VAS-F: Visual Analogue Scale for fatigue, pg/ml: picogram/milliliter).
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**De Pue Annelien**: Conceptualization, Methodology, Investigation, Resources, Writing – Review & Editing.

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