Natalizumab extended-interval dosing is associated with subtle inflammatory changes: a proof of concept study

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Background

Natalizumab is highly effective in reducing disease activity in people with MS (PwMS). Recently, extended-interval dosing (EID; i.e. 5-8 weeks interval) has proved comparable efficacy and reduced incidence of progressive multifocal leukoencephalopathy compared with standard-interval dosing (SID; i.e. 4 weeks interval). However, PwMS under EID frequently report fatigue and cognitive slowing towards the end-of-dose, which may reflect subtle changes in cortical excitability and inflammatory activity.

Our objective was to determine whether EID is associated with subtle inflammation. Our hypothesis was to detect cognitive slowing, a drop in peripheral pro-inflammatory lymphocytes, a change in the cortical GABAergic-glutamatergic tone toward higher excitability and an increase in neurofilaments.

Methods

This prospective longitudinal monocentric cohort study enrolled **25 PwMS under stable EID treatment (≥ 6 cycles) who underwent at the 2nd-4th week (intermediate visit) and at the 6th-8th week (end-of-dose visit) after Natalizumab infusion** a visit including the following:

Neuropsychological assessment: processing speed - **SDMT** (Symbol Digit Modalities Test), working memory - **PASAT** (Paced Auditory Serial Addition Task). *Self reported fatigue* - Fatigue Severity Scale (**FSS**).

Immunological assessment: Flow-cytometry analysis of the main **T-lymphocytes subsets and their cytokine production profile.**

Neurophysiological assessment: GABAergic and glutamatergic activity of the primary motor cortex were measured with Transcranial magnetic stimulation using paired-pulse stimulation. Short intracortical inhibition (**SICI,** intervals of 1 and 3 msec) is GABA-A dependent. Intracortical facilitation (**ICF,** intervals of 10 and 15 msec) is glutamatergic-dependent. We also measured the cortical silent period (**CSP**), to test GABA-B inhibitory mechanisms.

Plasma neurofilament dosage: the Simoa platform was used to quantify **plasma neurofilaments** (Quanterix Corp, Boston, MA). Plasma from 25 PwMS aged 35.32 (SD 8.76) with similar distribution of sex (60% female), disease duration (mean 10.72 years, SD 6), EDSS (median 1.5, IQR 1) and under stable SID natalizumab treatment (≥ 6 infusions every 4 weeks) were used for comparison.

Results

Neuropsychological assessment: **All the participants except one reported subjective end-of-dose symptoms, qualitatively described as fatigue and cognitive slowing worsening after the 4th week. However, we did not appreciate any significant change in the corresponding standardized measures.** In particular: FSS score did not increase at the end-of-dose (4.12, CI95% 3.43 – 4.8) compared with the intermediate visit (3.81, CI95% 3.16 – 4.46), despite 38.5% (10 subjects) of cases showed a clinically meaningful increase (i.e. ≥ 0.7 points).

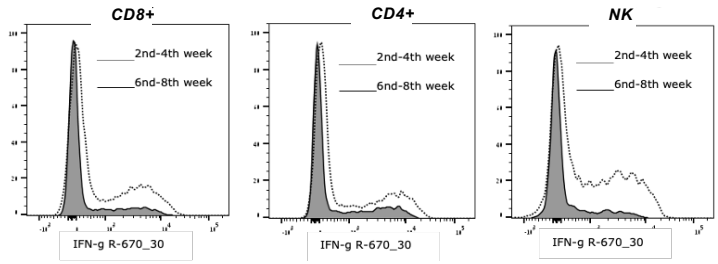
Immunological assessment: **As compared to the intermediate visit (2nd-4th week), at 6th-8th weeks after the last dosing we observed a substantial drop in the proportion of lymphocytes able to produce IFNγ and other pro-inflammatory subsets.** In particular, IFNγ-expressing lymphocytes almost halved at end-of-dose visit (CD4+: -57.7%, p=0.01; CD8+: -41.45%, p=0.014; NK: -54.23%, p=0.033, *Figure 1*). CD3+ γδ cells were significantly reduced considering both the overall cell count (-19.62%, p<0.001) and subtypes expressing perforin (-62.62%, p=0.009) or granzyme (-30.88%, p=0.013). Finally, the same trend was observed when looking at perforin-expressing lymphocytes (CD8+: -28.22%, p=0.017; NK: -3.37%, p=0.046) and activated CD69+ lymphocytes (CD4+: -26.9%, p>0.5; CD8+: -48.48%, p=0.046). **Other subsets, such as effector memory, central memory and naïve CD4+ and CD8+ lymphocytes, as well as the total number of NK cells did not change between the two time-points.**

Figure 1:

Pro-inflammatory lymphocytes drop in the peripheral circulation at the end-of-dose.

At the intermediate visit (ldotted line) there is a higher proportion of lymphocytes expressing IFNg, which are not present at the end-of dose visit (darker histogram).

The overlaid histograms graphically represent the expression of IFNg in lymphocyte subtypes after 4 hours re-stimulation with Ionomycin (1 µg/ml) and PMA (100 ng/ml).



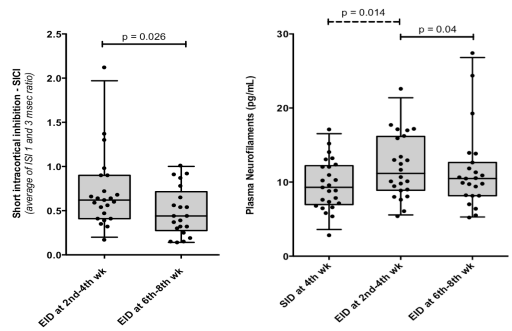
Neurophysiological assessment: **Intracortical short-latency inhibition (SICI), measured to test GABA-A activity, was reduced at the intermediate visit at the 2th-4th week compared to the 6th-8th week after the last infusion** (Figure 2A)**.** SICI was stronger at the end-of-dose (49%, CI95% 36% – 62%) compared with the intermediate visit (72%, CI95% 52% – 92%; F(1,20) 5.76; p=0.026, Cohen's d 0,63). The cortical silent period also decreased from 109.14 msec (CI95% 91.56 – 126.73) to 95.3 msec (CI95% 81.82 – 108.75), despite not reaching statistical significance. No significant changes were observed in ICF.

Plasma Neurofilaments Dosage: **Plasma neurofilaments were higher at the intermediate compared with the end-of-dose visit. The mean plasma neurofilaments in the EID cohort was higher than in a cohort of PwMS under SID treatment** (Figure 2B)**.** The mean level of plasma neurofilaments between the two visits was 12.08 pg/mL, CI95% 10.41–13.76, with higher values found at the intermediate (12.21 pg/mL, CI95% 10.22 – 14.2) compared with the end-of-dose visit (10.78 pg/mL, CI95% 8.85 – 12.72; F(1,21) 4.8; p=0.04, Cohen's d 0.32). Plasma from an unmatched sample of PwMS under stable SID obtained the 28th day after NTZ infusion showed lower NfL (9.38, CI95% 7.71–11.05) compared to the intermediate visit EID (F(1,47) 6.54; p=0.014, Cohen's d 0.69) and the mean NfL level in EID between the two timepoints (F(1,48) 5.28; p=0.026, Cohen's d 0.62).

Figure 2:

Fluctuation in cortical excitability and plasma neurofilaments during extended-interval dosing.

In people with MS under extended-interval dosing (EID) we found significant changes of cortical excitability and fluctuations in plasma neurofilaments. In particular (a), at the intermediate visit we found a reduction in the GABAergic tone in the primary motor cortex (SICI) as well as (b) a slight increase in plasma neurofilaments. The EID group showed higher levels of plasma neurofilaments at the 2nd-4th week compared with a control cohort of patients under standard interval (SID) at the 4th week (b). The boxplot shows the mean and the 5th to 95th percentile of distribution, p-values refer to paired sample T-test (continuous line) or unrelated sample T-test (dashed line).



Discussion

It is known that the infusion of NTZ is associated with an increase in pro-inflammatory lymphocytes in the peripheral circulation. We found an opposite effect comparing the end-of-dose with the intermediate visit. This may suggest the migration of pro-inflammatory lymphocytes to the CNS across a more permeable blood-brain barrier.

While end-of-dose symptoms reported by PwMS were vague and remain undetected by the standardized neuropsychological assessments used in the present study, they may represent a consequence of the increased level of pro-inflammatory cytokines. Pro-inflammatory cytokines in the CNS lead to a measurable reduction in cortical GABAergic activity, and neurofilaments are a sensitive marker of neurodegeneration. Both those measures showed significant fluctuations during the EID period.

Our a-priori study hypothesis was that a passage of pro-inflammatory lymphocytes across the blood-brain barrier would have resulted in a reduction in GABAergic function and an increase in neurofilaments. Despite both those changes were observed, their timing differs from our first hypothesis as we expected these changes to occur at the end-of-dose visit. Moreover, changes in NfL are below the effect size threshold of 0.5 for which this study was powered.

Nonetheless, prior literature supports the view that there might be a temporal gap between the passage of pro-inflammatory cells in the CNS and parenchymal damage, with NfL peaking over one month after blood-brain barrier's disruption.

The present study shows that EID is associated with changes in the peripheral immune profile and with fluctuations in cortical excitability and serum neurofilaments. Our findings hint at the possibility that subtle CNS inflammation may occur in EID and contribute to MS-related neurodegenerative phenomena.