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

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*Importance:* 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.

*Objective*: 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.

*Main outcome and measures:* The primary outcome was the change in peripheral lymphocyte subsets at the end-of-dose, the secondary outcomes were changes in: (i) neuropsychological tests of fatigue and cognitive speed, (ii) neurophysiological measures of cortical excitability associated with neuroinflammation, (iii) plasma neurofilaments.

*Design, settings and participants:* This prospective longitudinal monocentric cohort study enrolled 25 PwMS under stable EID treatment (≥ 6 cycles) who underwent at the $2^{nd}$-$4^{th}$ week (intermediate visit) and at the $6^{th}$-$8^{th}$ week (end-of-dose visit) after Natalizumab infusion an assessment of: (i) processing speed - PASAT, SDMT, (ii) self-reported fatigue - Fatigue Severity Scale, (iii) cortical GABAergic and glutamatergic activity, (iv) circulating lymphocytes subpopulations and cytokines production, (v) neurofilaments.

*Results:* All EID subjects but one reported end-of-dose symptoms, but no change in processing speed or self-reported fatigue was detected. We observed a substantial drop in the proportion of lymphocytes able to produce IFN$\gamma$ (CD4+: -57.7%, p=0.01; CD8+: -41.45%, p=0.014; NK: -54.23%, p=0.033) and other pro-inflammatory subsets at the end-of-dose. At the intermediate visit, we observed a reduction in the GABA-A dependent inhibition of the primary motor cortex (0.72, CI95% 0.52–0.92 vs 0.49, CI95% 0.36–0.61; $F_{(1,20)}$ 5.76; p=0.026), and a concomitant increase in plasma neurofilaments level (12.21 pg/mL, CI95% 10.22 – 14.2 vs 10.78 pg/mL, CI95% 8.85 – 12.72; $F_{(1,21)}$ 4.8; p=0.04).

*Conclusion and relevance:* We hypothesize that, at the end-of-dose, pro-inflammatory lymphocytes partially retrieve their competency to infiltrate the CNS, causing the observed fluctuations in cortical excitability, axonal damage, and conceivably vague end-of-dose symptoms.