PATTERNS OF REGIONAL GRAY MATTER AND WHITE MATTER ATROPHY IN PATIENTS STARTING FINGOLIMOD OR NATALIZUMAB: A 2-YEAR TENSOR-BASED MORPHOMETRY STUDY

[1,2]M.A. Rocca, [1,2]P. Preziosa, [1]G.C. Riccitelli, [2]M.E. Rodegher, [2]L. Moiola, [3]A. Falini, [2]G. Comi, [1,2]M. Filippi.

[1]Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, [2]Dept. of Neurology and [3]Dept.of Neuroradiology, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy.

INTRODUCTION and PURPOSE

Natalizumab (NAT) and fingolimod (FTY) are second-line treatments approved for patients with active relapsing-remitting (RR) multiple sclerosis (MS) and they have been proven to be highly effective in reducing clinical relapses, disability progression and active lesion formation [**1-10**]. Pivotal trials have shown the higher benefits of both FTY and NAT over placebo or interferon ß on clinical and MRI disease activity at two years. However, only a few observational studies have explored differences on clinical and MRI activity between the two drugs, with inconsistent results [**11-21**]. Additionally, the topographic patterns of longitudinal gray matter (GM) and white matter (WM) modifications after treatment initiation have been investigated for NAT only [**22-25**].

We compared the effects of FTY and NAT on preventing regional GM and WM atrophy in RRMS after two years of treatment.

METHODS

- **Study design**: Monocentric, prospective, longitudinal, open-label, non-randomized study.
- **Inclusion criteria**: (a) RRMS starting treatment with FTY or NAT, according to AIFA criteria; (b) Age ≥ 18 and ≤ 60 years; (c) EDSS ≤ 6.0; (d) Stable treatment from at least three months of other concomitant symptoms (e.g., fatigue, mood disturbances).
- **Esclusion criteria**: (a) Contraindications to MRI; (b) Other neurological or psychiatric diseases; (c) Major medical illnesses, including renal, hepatic or cardiac disease, or diabetes mellitus; (d) Pregnancy or breastfeeding.
- **Subjects**. Fifty-five RRMS patients starting NAT (n=30) or FTY (n=25). All patients underwent neurological and MRI assessments before starting treatment (T0), after six months (M6), one year (Y1) and two years (Y2) (+/- 7 days).
- **Neurological evaluation**: Rating of (a) clinical relapses, (b) EDSS, and (c) disability progression (EDSS score ≥1.0 point if baseline EDSS score was ≥1.0 or ≥ 1.5 points if the baseline score was 0).
- **Brain MRI acquisition**: 3.0 Tesla scanner: (a) dual-echo turbo spin-echo (TSE), (b) 3D T1-weighted fast field echo (FFE), and (c) post-gadolinium (Gd) T1-weighted scans.
- **MRI analysis**:
- Quantification of number of Gd-enhancing lesions at T0, M6, Y1 and Y2 and evaluation of number of new T2-hyperintense WM lesions at M6, Y1 and Y2 (*Jim 6.0, Xinapse System*).
- Estimation of T2-hyperintense lesion volumes (LVs) at T0, M6, Y1 and Y2 (*Jim 6.0, Xinapse System*).
- Quantification of normalized brain volume (NBV) at T0 and percent brain volume changes (PBVC) (*SIENAx* and *SIENA*).
- **Mapping regional GM and WM volumes changes**:
- *Voxel-Based Morphometry (VBM) (T0) (SMP12, DARTEL)*: Transformation of GM and WM maps, obtained from segmentation, to MNI space, non linear deformation of GM/WM maps to match the final customized template, modulation to keep original volume unchanged, and smoothing (8 mm gaussian kernel).
- *Tensor-Based Morphometry (TBM) (longitudinal changes) (SPM12, Serial Longitudinal registration, DARTEL)*: Groupwise alignment among each of the subject's scans, production of a mid-point average template, evaluation of the evolution of the Jacobians at the different timepoints, and normalization to MNI space [**26-27**].
- **Statistical analysis**:
- Non-parametric test for equality of median, Fisher's exact test, Chi-Square test and hierarchical mixed model adjusted for previous treatment: comparison of demographic, clinical and MRI measures between FTY- and NAT-groups.
- VBM and TBM (SPM12) (p<0.05 FWE corrected):
  - Input images: GM and WM tissues (VBM), difference between pairs of Jacobians (TBM).
  - Within-group and between group comparisons: one sample and analysis of covariance (ANCOVA), using age and gender as covariates.

RESULTS

Table 1 shows the main baseline demographic, clinical and MRI characteristics of in the two cohorts of RRMS patients starting FTY or NAT.

Table 1.

Variables	FTY (n=25)	NAT (n=30)	p value
Women/Men	15/10	18/12	n.s.^
Median age (range) [years]	38.3 (19.2,53.2)	36.5 (21.6,56.9)	n.s.*
Median DD (range) [years]	10.3 (2.0,25.6)	8.2 (0.5,23.0)	n.s.*
Median EDSS score (range)	2.0 (1.0,5.5)	2.0 (1.0,6.0)	n.s.*
Mean ARR in the previous year (range)	1.00 (0,3)	1.20 (0,3)	n.s.*
Mean ARR in the previous two years (range)	0.88 (0,5)	0.82 (0,4)	0.77*
Last treatment before recruitment None / 1st line DMD / FTY / NAT / Immunosuppressants	0 / 18 / 0 / 6 / 1	4 / 23 / 2 / 0 / 1	0.003#
Median T2 LV (range) [ml]	6.3 (0.6,38.7)	5.1 (0.6,47.3)	n.s.*
Median Gd-enhancing lesion number (range)	0 (0,2)	0 (0,2)	n.s.*
Number (%) of patients free of Gd-enhancing lesions	21 (84.0%)	22 (73.3%)	n.s.#
Median NBV (range) [ml]	1511 (1300,1678)	1530 (1250,1711)	n.s.*

^=Chi-Square Test *= Non-parametric Test for equality of median #=Fisher's exact Test

Clinical findings:

- Stabilization of EDSS score for both drugs at each timepoint, with a significant improvement of EDSS score in FTY at Y1 *vs* T0 (-0.20, p=0.02).
- Reduction of ARR after treatment initiation for both drugs compared to the year before treatment initiation (FTY=0.32 at M6, 0.24 at Y1, 0.12 at Y2; NAT=0.00 at M6, 0.03 at Y1, 0.02 at Y2, p<0.0001 for all comparisons) and in NAT *vs* FTY at M6 (p=0.02);
- Similar number of relapse-free patients in NAT *vs* FTY at M6 (100% *vs* 84.0%, p=0.18) and a trend for a higher number of relapse-free patients in NAT *vs* FTY at Y1 and Y2 (96.7% *vs* 76.0%, p=0.06 for both timepoints);
- No significant difference in disability progression at Y2 between the to drugs (0% in FTY *vs* 6.7% in NAT, p=0.11).

MRI findings: **Table 2** shows the main longitudinal MRI changes during the follow-up in two cohorts of RRMS patients.

Table 2.

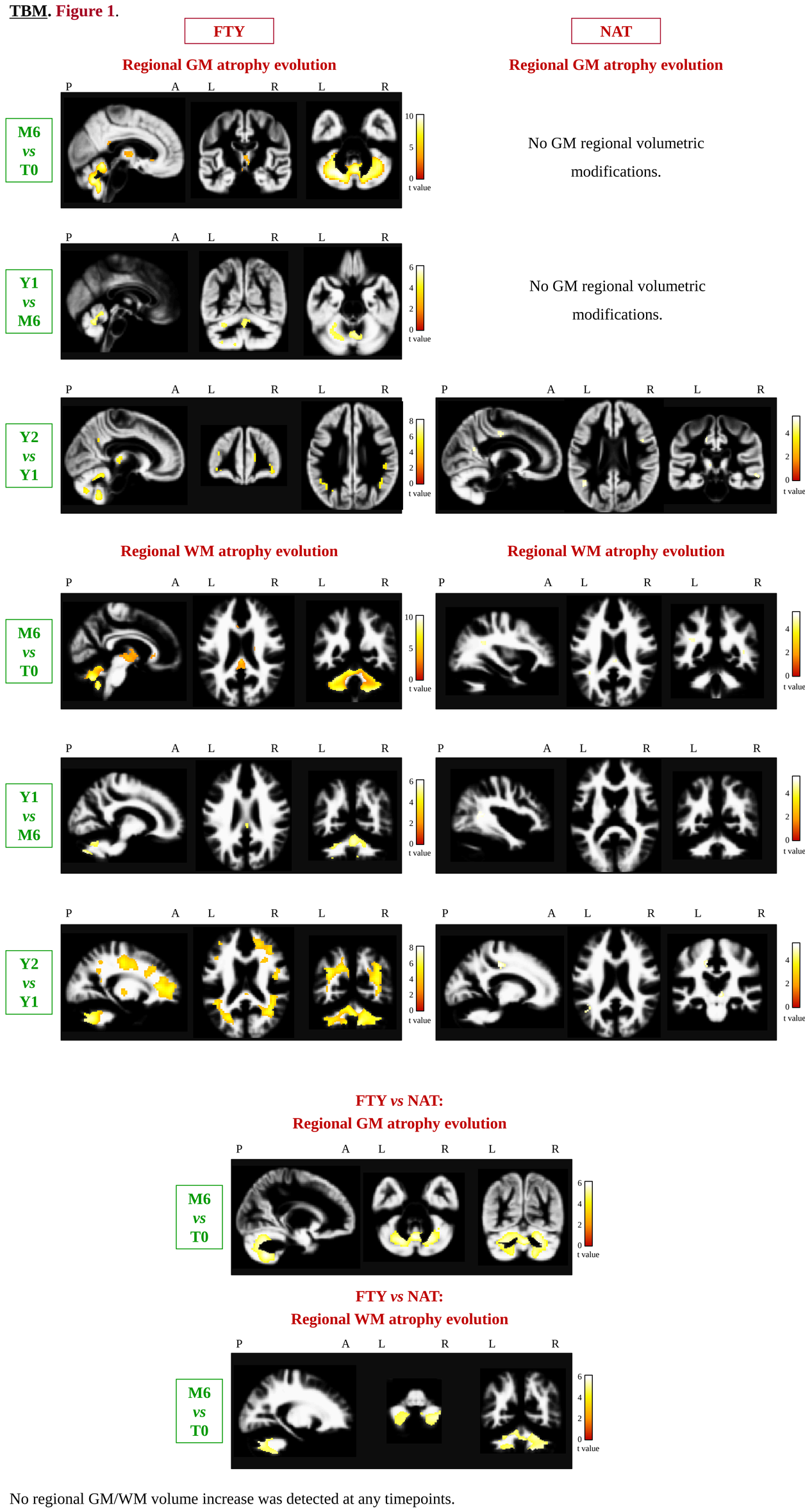
Variables		FTY (n=25)	NAT (n=30)	p value*
Median number of new T2 lesions (range)				
	M6-T0	0 (0, 5)	0 (0, 6)	n.s.
	Y1-M6	0 (0, 2)	0 (0, 1)	0.02
	Y2-Y1	1 (0, 6)	0 (0, 7)	n.s.
Number (%) of patients free of new T2 lesions				
	M6-T0	13 (52.0%)	21 (70.0%)	0.05
	Y1-T0	10 (40.0%)	21 (70.0%)	0.003
	Y2-T0	9 (36.0%)	20 (66.7%)	0.003
Median number of Gd-enhancing lesions (range)				
	M6	0 (0, 1)	0 (0, 0)	n.s.
	Y1	0 (0, 1)	0 (0, 0)	n.s.
	Y2	0 (0, 1)	0 (0, 0)	n.s.
Number (%) of patients free of Gd-enhancing lesions				
	M6-T0	24 (96.0%)	30 (100.0%)	n.s.
	Y1-T0	23 (92.0%)	30 (100.0%)	n.s.
	Y2-T0	23 (92.0%)	30 (100.0%)	n.s.
Median PBVC (range)				
M6-T0	p value*	-0.20 (-1.65,0.57) 0.03	-0.25 (-1.28,0.51) 0.003	n.s.
Y1-M6	p value*	-0.03 (-0.68,0.73) n.s.	-0.07 (-0.93,0.56) 0.04	n.s.
Y2-Y1	p value*	-0.30 (-1.18,0.53) 0.001	-0.22 (-1.18,0.53) 0.009	n.s.

*=Hierarchical mixed model analysis adjusted for previous treatment

- Compared to T0, FTY patients showed a significant increase of T2-hyperintense LVs at each timepoint (p values<0.001 for all comparisons), whereas NAT patients showed a significant decrease of T2-hyperintense LVs at Y2 (p=0.01).

VBM at T0. At T0, no GM nor WM volume difference was found between FTY and NAT patients.

TBM. Figure 1.



No regional GM/WM volume increase was detected at any timepoints.

CONCLUSIONS

- **FTY and NAT are highly effective in reducing clinical relapses and MRI activity and preventing disability progression after 2 years of treatment in RRMS, with a slight superiority of NAT.**
- **Regional GM atrophy occurred already at M6 and progressed during the subsequent timepoints in FTY group, mainly involving the cerebellar cortex, but also some cortical and subcortical structures, while NAT-patients showed a significant atrophy of some clusters in cortical and subcortical regions only at Y2 *vs* Y1.**
- **Regional WM atrophy occurred for both treatments already at M6 and then progressed at Y1 and Y2, involving both infratentorial and supratentorial WM tracts, with a significant higher cerebellar WM atrophy in FTY compared to NAT.**
- **The strong anti-inflammatory effects of NAT might promote a secondary neuroprotection through a reduction of further inflammatory processes and the development of a more favourable environment to enhance tissue recovery, allowing a more significant effect on preventing regional irreversible tissue loss.**
- **FTY might reduce neuroinflammation and exert direct neuroprotective effects on different CNS cells, including oligodendrocytes, astrocytes, and neurons, but with possible regional differences in the effectiveness these mechanisms.**
- **Further studies with larger sample size and longer follow-up are warranted to confirm these results and to better understand the pathophysiologic mechanisms influencing the different pattern of GM and WM atrophy related to these treatments.**

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