EFFECTS OF FINGOLIMOD AND NATALIZUMAB ON SLOWLY EXPANDING LESION OCCURRENCE OVER TWO YEARS OF TREATMENT IN RELAPSING-REMITTING MULTIPLE SCLEROSIS

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INTRODUCTION and PURPOSE

Pathological studies demonstrated the presence of chronic active or mixed (active and inactive) lesions in multiple sclerosis (MS) that are typified by a "rim" of iron-laden activated microglia and/or macrophages and a slow rate of ongoing demyelination and axonal loss [1-4]. Such smouldering inflammation can occur in lesions showing a slow rate of increase in size and ongoing tissue loss over time and contribute to disability progression [1-4]. Natalizumab (NAT) and fingolimod (FTY) are second-line treatments approved for patients with active relapsing-remitting (RR) MS and they have been proven to be highly effective in reducing disease activity in terms of clinical relapses and acute lesion formation [5-14]. However, their ability to limit smouldering inflammation has not been evaluated yet.

We compared the effects of these drugs on the occurrence of white matter (WM) lesions showing a progressive linear enlargement over 2 years of treatment, thus defined slowly expanding lesions (SELs) and representing a putative biomarker of smouldering inflammation.

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) Expanded Disability Status Scale (EDSS) score ≤ 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. RRMS patients starting NAT (n=30) or FTY (n=25). All patients underwent neurological and MRI assessments before starting treatment (T0), after 6 (M6), 12 (M12) and 24 months (M24) (+/- 7 days).

Neurological evaluation. Rating of (a) clinical relapses, (b) annualized relapse rate (ARR), (c) EDSS, and (d) 3-month confirmed disability progression (3-month-CDP) (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), (c) 3D T1-weighted FFE with and without of resonance pulses applied, and (d) post-gadolinium (Gd) T1-weighted scans.

Conventional MRI analysis.

- Quantification of number of Gd-enhancing lesions at T0, M6, M12 and M24 and evaluation of number of new T2-hyperintense WM lesions at M6, M12 and M24 (*Jim 6.0, Xinapse System*).
- Estimation of T2-hyperintense lesion volumes (LVs) and creation of T2-hyperintense lesion masks at T0 (*Jim 6.0, Xinapse System*) (**Figure 1**).

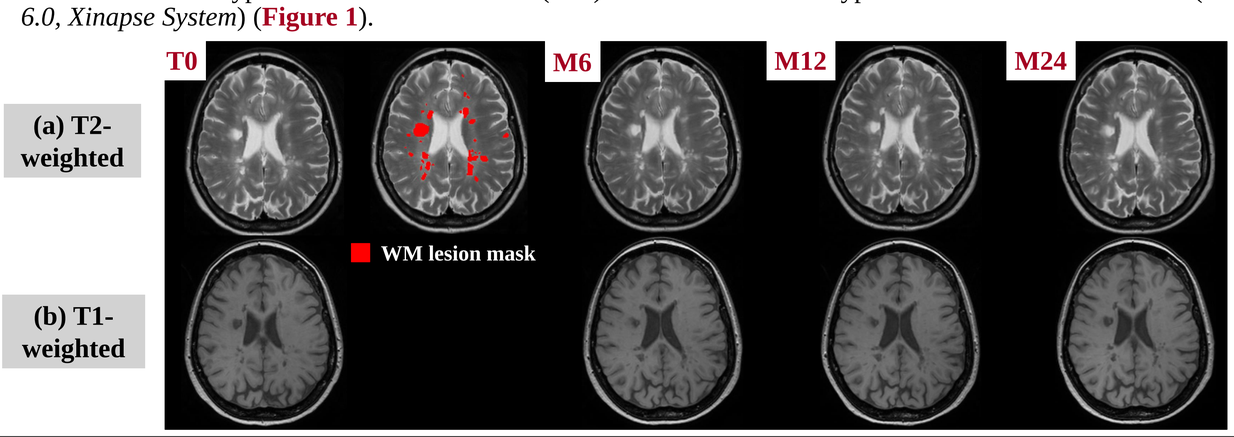

Figure 1. Example of axial slices of (a) T2-weighted and (b) T1-weighted sequences acquired at the different scheduled timepoints of the study. Mask of baseline T2-hyperintense WM lesions is shown in red.

SELs analysis. SELs were investigated using an in-house implemented method based on that proposed by Elliott et al. [15]. SELs were identified among baseline T2-hyperintense lesions, by linearly fitting the Jacobian of the non-linear deformation field between timepoints, obtained using T1- and T2-weighted scans. A threshold ≥10% of annual increase was applied and neighbour voxels were grouped in clusters. Total number of lesions and the percentage of SELs, their volumes and the average magnetization transfer ratio (MTR) values were calculated considering clusters ≥10 voxels (**Figure 2**).

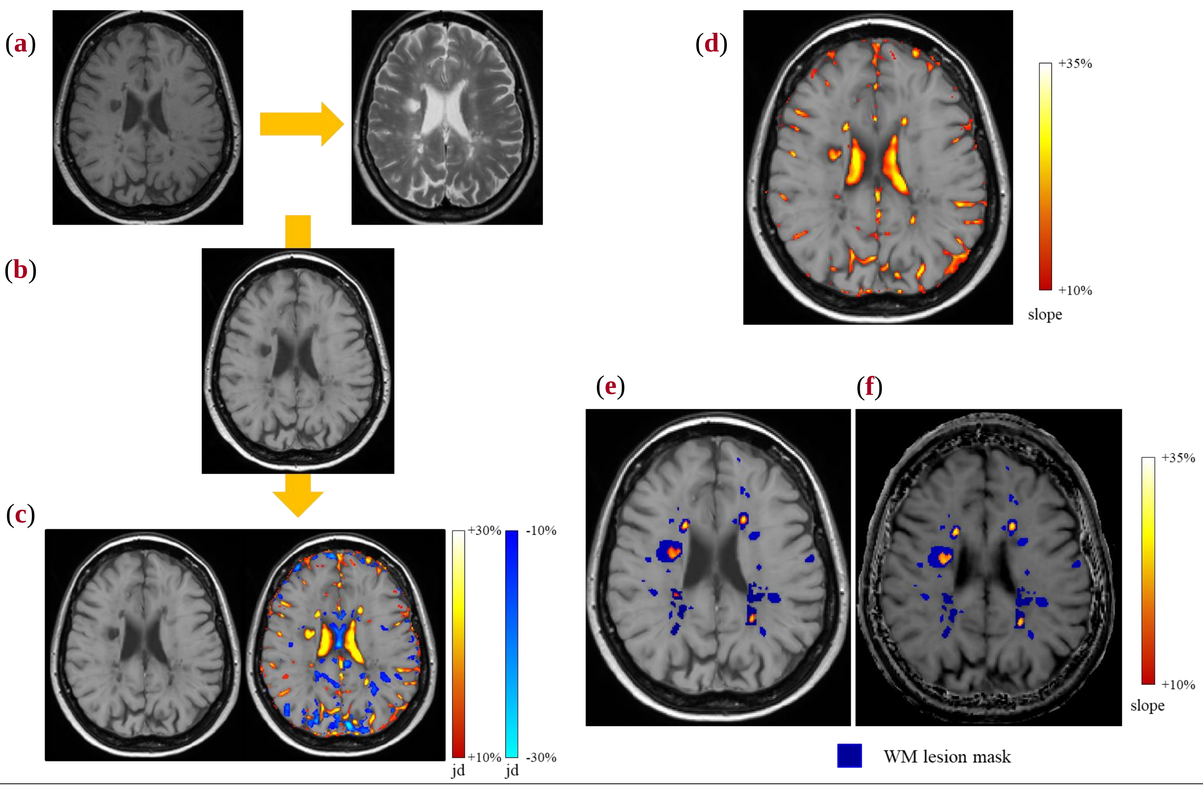

Figure 2. Schematic representation of the methods applied to identify slowly-expanding lesions (SELs). (**a**) Resampling of 3D T1-weighted sequence to T2-weighted sequence. (**b**) Creation of combined image (CI) from T1- and T2-weighted sequences [16] and resampling back to T1-weighted sequence. (**c**) Longitudinal registration of the four timepoints to an average template (SPM12) and quantification of the percentage Jacobian differences (jd) vs T0. (**d**) Linear fitting of the slopes of Jd, application of a threshold ≥10% of annual increase and grouping of neighbour voxels into clusters. (**e**) Quantification of total number and volumes of SELs. (**f**) Quantification of magnetization transfer ratio (MTR) values in SELs + and SELs -.

Statistical analysis. Comparison of baseline demographic, clinical and MRI measures and within- and between-group longitudinal changes were performed using Mann-Whitney, Pearson chi-square, Fisher exact or Wilcoxon Signed Rank tests as appropriate.

RESULTS

The two cohorts of RRMS starting FTY or NAT were matched for the main demographic, clinical and conventional MRI findings (**Table 1**).

Variables	FTY (n=25)	NAT (n=30)	p-value
Women/Men	15/10	18/12	n.s.^
Mean age (SD) [years]	37.5 (8.7)	36.8 (10.2)	n.s.*
Median disease duration (IQR) [years]	10.3 (5.4;15.5)	8.2 (4;14.8)	n.s.*
Median EDSS score (IQR)	2.0 (1.5;3.0)	2.0 (1.5;4.0)	n.s.*
Mean ARR in the previous year (range)	1.00 (0,3)	1.20 (0,3)	n.s.*
Last treatment (%): None/First line/Second line	0/18/7	4/23/3	0.06#
Median T2 lesion number (IQR)	75 (40-114)	54 (29-91)	n.s.*
Median T2 LV (IQR) [ml]	4.0 (2.0;9.2)	3.0 (1.2;8.7)	n.s.*
Median Gd(+) lesion number (IQR)	0 (0;0)	0 (0;1)	n.s.*

^Chi-Square Test; *Mann-Whitney Test; #Fisher's exact Test.

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

Longitudinal clinical and MRI findings.

- Both treatments significantly reduced the ARR (mean ARR=0.12 with FTY and 0.02 with NAT, p-value<0.001 for both) , with a significant superiority of NAT (p=0.02), and promoted a high prevalence of patients free from clinical relapses (FTY=76%; NAT=97%), without significant differences between groups.
- Both treatments stabilized EDSS, without significant differences from 3-month CDP (FTY=100%; NAT=93%, p=0.31).
- Both patients' groups showed a significant accumulation of new T2-hyperintense lesions (mean=1.92, p-value<0.001 in FTY; mean=0.83, p-value=0.02 in NAT), with NAT patients having a lower accumulation of new T2-hyperintense lesions (p=0.03), and a higher prevalence of freedom from MRI activity (64% *vs* 33%, p=0.02).
- At M24, FTY patients showed an increase in T2-hyperintense LV (p<0.001), while a decrease in T2-hyperintense LV was found for NAT patients (p<0.001).

SELs analysis.

The proportion of FTY-patients showing ≥1 SEL was higher compared to NAT (96% *vs* 50%, p<0.001). Compared to natalizumab-patients, fingolimod-patients showed a higher mean number (6.44 *vs* 3.40, p=0.004), and volume (0.20 *vs* 0.13 ml, p=0.002) of SELs, and mean percentages of lesions (7.30% *vs* 4.32%, p=0.007) and of lesional volume (2.12% *vs* 0.92%, p<0.001) defined as SELs (**Figure 3**).

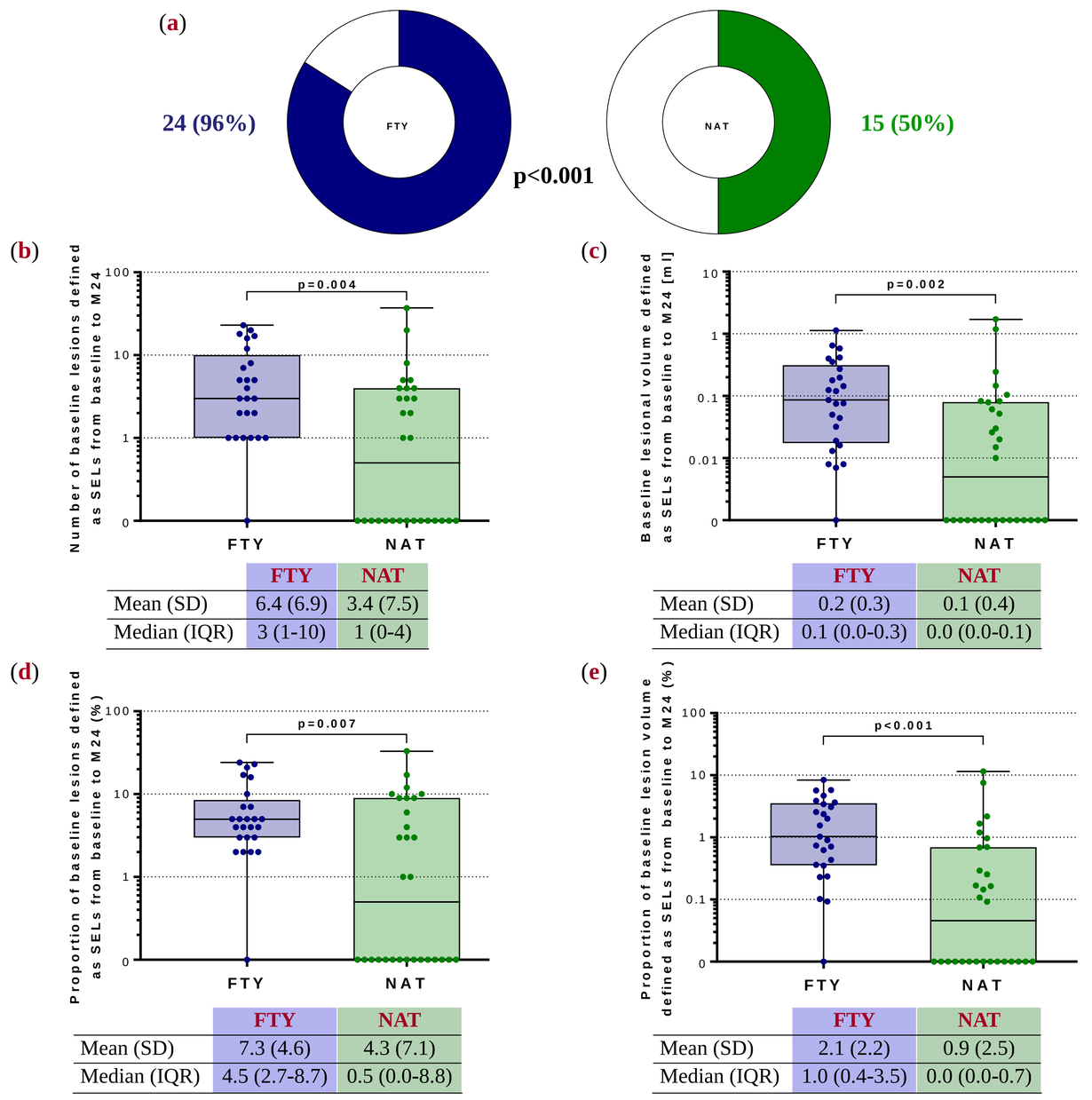

Figure 3. (**a**) Proportion of RRMS patients showing ≥1 baseline lesion defined as SEL from baseline to M24. (**b**) Total number of baseline lesions per patient defined as SELs from baseline to M24. (**c**) Total baseline lesional volume defined as SELs from baseline to M24. (**d**) Proportion of baseline lesions defined as SELs from baseline to M24. (**e**) Proportion of baseline lesion volume defined as SELs from baseline to M24.

MTR analysis.

In both groups, compared to SELs -, SELs + showed significantly lower mean MTR values at T0 (0.27 vs 0.34 in FTY-group; 0.27 vs 0.34 in NAT-group, p<0.001), with no significant between-group differences and longitudinal changes (**Figure 4**).

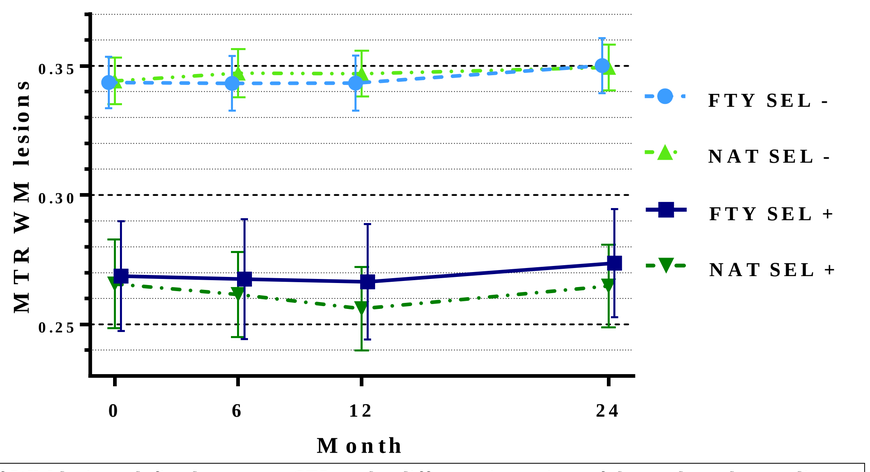

Figure 4. Comparisons of MTR values of WM lesions defined or not as SEL at the different timepoints of the study and according to treatment.

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.**
- **SELs assessment using T1- and T2-weighted sequences is feasible in MS patients. Such an approach could allow to identify chronic active lesions characterized by smouldering inflammation, ongoing demyelination and axonal loss and could provide useful information to monitor the therapeutic effects of FTY and NAT on this pathological substrate.**
- **MTR contributes to identify WM lesions showing a more severe microstructural damage, which are characterized by a slow but progressive increase in size and ongoing tissue loss over time.**
- **Our study suggests a stronger effect of NAT in limiting the number and volume of SELs, possibly through its strong anti-inflammatory activity.**
- **NAT and FTY are likely to similarly prevent the accumulation of microstructural tissue damage in both SELs and not-SELs, possibly through the prevention of further inflammation, the development of a more favourable environment to enhance tissue recovery.**
- **Further studies with larger sample size and longer follow-up are warranted to confirm these results and to better understand the effects of these treatments on SELs occurrence and the influence on disability progression.**

LITERATURE

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