

ORIGINAL ARTICLE

Longitudinal stability of progression-related microglial activity during teriflunomide treatment in patients with multiple sclerosis

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Abstract

Background and purpose: The aim was to study brain innate immune cell activation in teriflunomide-treated patients with relapsing–remitting multiple sclerosis.

Methods: Imaging with 18-kDa translocator protein positron emission tomography (TSPO-PET) using the [¹¹C]PK11195 radioligand was employed to assess microglial activity in the white matter, thalamus and areas surrounding chronic white matter lesions in 12 patients with relapsing–remitting multiple sclerosis who had been treated with teriflunomide for at least 6 months before inclusion. Magnetic resonance imaging (MRI) was used to measure lesion load and brain volume, and quantitative susceptibility mapping (QSM) was used to detect iron rim lesions. These evaluations were repeated after 1 year of inclusion. Twelve age- and gender-matched healthy control subjects were imaged for comparison.

Results: Half of the patients had iron rim lesions. In TSPO-PET, the proportion of active voxels indicating innate immune cell activation was slightly greater amongst patients compared with healthy individuals (7.7% vs. 5.4%, $p=0.033$). The mean distribution volume ratio of [¹¹C]PK11195 was not significantly different in the normal-appearing white matter or thalamus amongst patients versus controls. Amongst the treated patients, no significant alteration was observed in positron emission tomography distribution volume ratio, the proportion of active voxels, the number of iron-rim-positive lesions, lesion load or brain volume during follow-up.

Conclusions: Compared to controls, treated patients exhibited modest signs of diffuse innate immune cell activity, which was unaltered during follow-up. Lesion-associated smoldering inflammation was negligible at both timepoints. To our knowledge, this is the first study applying both TSPO-PET and QSM-MRI to longitudinally evaluate smoldering inflammation.

KEYWORDS

magnetic resonance imaging, multiple sclerosis, positron emission tomography, quantitative susceptibility mapping, teriflunomide

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INTRODUCTION

Multiple sclerosis (MS) is a leading cause of disability in younger adults in the Western world [1, 2], and microglial activation is a hallmark of progression-related MS pathology. Chronic slowly expanding lesions are characterized by a perilesional ring of activated microglia, whilst inactive plaques contain low numbers of innate immune cells or other inflammatory cells [3, 4]. Quantitative susceptibility mapping (QSM) is an experimental magnetic resonance imaging (MRI) method able to capture susceptibility changes caused by, for example, iron deposition within microglia [5], and iron containing CD68+ microglia and macrophages have been identified around QSM-positive MS lesions with immunohistochemistry [6].

Used in over 500 patients with MS, positron emission tomography (PET) imaging utilizing the 18-kDa translocator protein (TSPO) binding radioligand [^{11}C]PK11195 is an established in vivo method to monitor smoldering inflammation in the MS brain [7, 8]. Whilst a subset of astrocytes and endothelial cells may contribute to the TSPO signal, microglia and macrophages constitute the main source of TSPO within the normal-appearing white matter (NAWM) and at the edge of lesions [9–11].

In addition to correlating with disability, as measured with the Expanded Disability Status Scale (EDSS) [12], [^{11}C]PK11195 uptake predicts the development of clinically definite MS [13] and disease progression independent of relapse [14]. Interventions with disease-modifying treatments (DMTs) reduce TSPO binding at chronic MS lesions [15, 16] and also in the NAWM [15]. By linking disability progression to microglial activation in the NAWM [17], TSPO-PET has been instrumental in expanding the investigational scope of the pathophysiological process beyond conventional lesions seen with MRI.

Teriflunomide is a treatment for relapsing–remitting MS (RRMS) first introduced almost a decade ago. Of interest is its efficacy in preventing EDSS-measured disability progression in two phase III trials in patients with relatively active MS [18, 19]. As the more recent real-world use of teriflunomide has concentrated on milder MS, this non-interventional study enrolled patients at risk of disease progression based on relatively advanced age, which is one of the strongest known predictors of progression [20]. Patients with secondary progressive MS (SPMS) exhibited increased microglial activation compared with patients with RRMS [21]. Thus, it was of interest to assess TSPO binding in a real-world non-interventional setting of first-line DMT use amongst middle-aged patients at progression risk.

MATERIALS AND METHODS

Study design

This was an open-label, non-interventional study performed at the Turku University Hospital Neurocenter and Turku PET Centre, part of the University of Turku, and Turku University Hospital. Although the study was non-interventional regarding teriflunomide treatment,

it was interventional regarding TSPO-PET imaging. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland. The study was registered in clinicaltrials.gov (NCT03368677; <https://www.clinicaltrials.gov/ct2/show/study/NCT03368677>). Study participants provided written informed consent according to the principles of the Declaration of Helsinki, after which they underwent baseline neurological assessments and MRI and TSPO-PET scans within approximately 1 month of inclusion. These baseline assessments and scans were repeated approximately 1 year after the initial PET visit.

Study subjects

Sixteen eligible subjects treated with teriflunomide in the Hospital District of Southwest Finland were recruited between December 2017 and December 2020. Patients with RRMS, who were treated with teriflunomide for at least 6 months before inclusion, between 40 and 55 years of age, with EDSS score between 1.0 and 6.5 and at least nine T2 lesions at baseline MRI, were considered for the study. Key exclusion criteria included pregnancy, serious liver disease and other significant central nervous system (CNS) pathology besides MS. One subject suffered a panic attack in the PET camera and withdrew her consent after the baseline visit. Another subject discontinued teriflunomide due to poor perceived tolerability and was subsequently excluded between visits. Fourteen subjects remained for the entire study duration. One patient was later diagnosed with SPMS from before enrollment and was excluded from the analysis, and another subject was excluded due to failed radioligand synthesis resulting in inadequate injected activity at follow-up imaging. The temporary loss of follow-up resulted in an extended interval of >2 years between the PET scans for one subject, but these data were included in the analysis as an extended follow-up was not expected to produce significant bias. For comparison, 12 age- and gender-matched healthy individuals were imaged at baseline using TSPO-PET and MRI.

Magnetic resonance imaging and image processing

All subjects underwent 3-T MRI (Philips Ingenia/Philips Ingenuity, Best, The Netherlands) at baseline and after 1 year of follow-up with the following sequences: T1, T2, fluid-attenuated inversion recovery (FLAIR) and 3D high resolution T1. The PET images were co-registered with the respective T1 images for each subject using statistical parametric mapping (SPM8) (Wellcome Trust Center for Neuroimaging, London, UK). Preliminary lesion masks with variable thresholds were first created using the Lesion Segmentation Toolbox (LST) [22] in SPM, after which these preliminary masks were checked and edited to correspond to T1-hypointense chronic MS lesions according to visual inspection. A chronic lesion was defined as measuring >3 mm³ voxels in at least one dimension with the most hypointense voxels below the intensity of normal gray matter.

The perilesional mask used to quantify tracer uptake surrounding chronic lesions was created by dilating the lesion mask by 3 mm and then subtracting the core image from the 3-mm image.

The LST was used to create FLAIR lesion masks for each patient's baseline MRI, and the resulting masks were manually checked for segmentation errors and edited, if necessary, to also include abnormal white matter FLAIR signals in the perilesional area. The masks were then used on the 1-year follow-up images and edited to account for any visible change in lesion volume. Finally, the T1 and FLAIR masks were combined and subtracted from each patient's segmented white matter masks created with LST's lesion-filling tool similar to that described in Sucksdorff et al. [15] to create NAWM masks. The T1 lesion mask images were also used to fill the corresponding T1 image with the LST lesion-filling tool. The filled T1 was then used to segment thalamic volumes with FreeSurfer (<https://surfer.nmr.mgh.harvard.edu/>) for PET assessments.

To estimate rater-independent changes in T2 lesion volume, *nicMS*lesions [23] was first used to segment the lesions from baseline FLAIR images; then the longitudinal pipeline of *nicMS* was employed using the baseline masks as input. The threshold was set to ≥ 9 voxels to exclude small unspecific T2 hyperintensities of < 3 mm in any dimension. Whole brain volume normalized for head size was estimated with SIENAX [24], and thalamic volumes were segmented with FSL FIRST [25].

Iron rim detection using QSM

The QSM images were processed using the Morphology Enabled Dipole Inversion toolbox with automatic uniform cerebrospinal fluid zero reference (MEDI+0) [26] from the multi-echo gradient echo sequence data. The reconstructed QSM images were then co-registered with the T1 images using SPM12. Lesions manifesting a positive susceptibility value indicating iron were preselected. If the QSM signal was morphologically consistent with a lesion-associated ring, this lesion was determined as a QSM iron rim lesion by visual inspection by two experienced raters. Additionally, ITK-SNAP (<http://www.itksnap.org/pmwiki/pmwiki.php>) was used to quantify the signal intensity in the perilesional area and to ascertain the relative hypointensity of the respective core.

^{11}C PK11195 radioligand production and PET

To produce the radioisotope ^{11}C , irradiations were performed with a TR-19 (ACSI, Richmond, Canada) cyclotron by proton bombardment of ^{14}N utilizing the $^{14}\text{N}(p, \alpha)^{11}\text{C}$ nuclear reaction. A target gas mixture of 0.2% oxygen in natural nitrogen produced radioactive CO_2 . Fifteen minutes of irradiation (19 MeV, 40 μA , 41 bar, 20°C) yielded 60 GBq of ^{11}C CO₂.

Detailed synthesis steps to produce the radiochemical compound ^{11}C PK11195 from its precursor have been described elsewhere [27, 28]. Briefly, ^{11}C PK11195 was obtained by trapping ^{11}C

CH_3I at room temperature into a reaction vessel containing 1.0 mg of precursor (R)-*N*-desmethyl-PK11195 (1-(2-chlorophenyl)-*N*-(1-methylpropyl)-isoquinoline-3-carboxamide) and 8-quinolinol (1 M, 3 μl) in dimethylsulfoxide (200 μl). The reaction mixture was diluted with the high performance liquid chromatography (HPLC) mobile phase (500 μl) before injection into the HPLC system with a Phenomenex Luna column (C18(2), 10 \times 250 mm, 10 μm). Acetonitrile/0.01 M H_3PO_4 (65/35, v/v) was used as the HPLC mobile phase with a flow rate of 6 ml/min. The product was further purified by SPE cartridge (SepPak C18 light, Waters) and formulated with ethanol (1 ml), phosphate buffer (0.1 M, 8.5 ml) and propylene glycol (1.5 ml).

Sixty minutes of dynamic list mode PET data were acquired twice for each subject with a high resolution research tomograph (Siemens Medical Solutions, Knoxville, TN, USA). At baseline, the mean \pm standard deviation (SD) injected dose of radioactivity was 462 ± 73 MBq for the teriflunomide-treated patients with MS, and 486 ± 17 MBq for the age-matched controls. At 1-year follow-up, the injected dose of radioactivity was 480 ± 52 MBq for teriflunomide-treated patients. No significant dose differences between the groups or timepoints were observed.

Positron emission tomography data processing and analysis

Image reconstruction was performed with the OP-OSEM algorithm [29] using 17 timeframes. PET image post-processing followed a previously described procedure [15]. Briefly, the dynamic images were smoothed, realigned and co-registered using SPM8. Images were then resliced to match the 1-mm voxel size of the MRI images.

Specific binding of ^{11}C PK11195 was quantified using distribution volume ratios (DVRs). As no reliable anatomical region devoid of activated microglia (i.e., specific PK11195 binding) exists, time-activity curves representing a region without specific binding were acquired with the MATLAB (MathWorks Inc., Natick, MA, USA) software Super-PK using a supervised cluster algorithm [30] optimized with four predefined kinetic tissue classes [31]. The Logan variant reference tissue model [32] with a 20- to 60-min time interval was applied to the regional time-activity curves.

To determine the proportions of individual active voxels, the mean DVR (\pm SD) of all voxels in the NAWM of healthy control subjects was first calculated. A 95% confidence threshold was determined with the formula $\text{mean} + 1.96 \times \text{SD}$, which was used for dichotomous classification of each voxel of all subjects, that is, values above the threshold signified an active voxel. Based on our previous data from healthy controls [33], this threshold was set to 1.56. Clusters below the three connected voxels were excluded to prevent the inclusion of random peak values.

Parametric DVR maps of one subject's baseline and follow-up lesion masks dilated 3 mm in each direction were laid on top of T1 MRI images from the respective timepoints using Mango (<https://mangoviewer.com/>) to produce an illustration of perilesional ^{11}C PK11195 binding. Surface plots from individual lesions were created using Fiji (<https://imagej.net/software/fiji/>).

Analysis of chronic lesion subtypes according to microglial activation

Lesion phenotyping was performed according to Nylund et al. [33]. Briefly, individual lesions in the T1 lesion masks were classified into “inactive”, “overall active” and “rim active” based on the presence and distribution of active voxels in the core versus rim. Chronic T1 lesions with a minimum volume of 27 mm³ were considered.

Statistical analysis

The statistical analysis was performed using R version 4.1.1 (<https://www.r-project.org/about.html>) and SAS version 9.4 (SAS Institute Inc.). The Wilcoxon rank-sum test was used for comparisons between controls and patients, and the Wilcoxon

signed-rank test was used to compare the baseline and follow-up measurements of the patients. Gender comparisons were performed using Fisher's exact test. All tests were two-tailed, and *p* values less than 0.05 were considered as statistically significant for all analyses.

RESULTS

Study subjects

The teriflunomide-treated cohort (*n*=12) consisted of three males and nine females of Caucasian descent with an average (\pm SD) age and disease duration of 46.1 \pm 6.2 and 10.8 \pm 8.1 years, respectively. The mean (\pm SD) duration of teriflunomide treatment was 10 \pm 3.5 months before baseline imaging. The demographic baseline characteristics are listed in Table 1.

TABLE 1 Demographics and lesion phenotyping

ID (M/F)	Timepoint	Disease duration	EDSS	ARR before inclusion	Rim active	Overall active	Inactive	QSM+
P01 (F)	Baseline	12.12	1.5	0.25	0	0	2	0
	Follow-up		2.5		0	2	0	0
P02 (F)	Baseline	6.43	3.5	0.16	1	2	8	0
	Follow-up		3.0		0	3	8	0
P03 (F)	Baseline	3.56	1.5	0.28	0	4	1	2
	Follow-up		2.0		2	1	2	2
P04 (F)	Baseline	7.29	2.5	0.14	1	3	1	2
	Follow-up		4.0		1	2	3	2
P05 (F)	Baseline	12.56	2.0	0.32	1	4	2	1
	Follow-up		2.0		1	4	2	1
P06 (F)	Baseline	21.64	2.5	0.32	1	2	5	1
	Follow-up		2.5		1	2	5	1
P07 (F)	Baseline	7.89	2.0	0.25	0	2	2	2
	Follow-up		2.0		1	4	0	2
P08 (F)	Baseline	29.83	3.5	0.70	0	0	2	0
	Follow-up		3.5		0	1	1	0
P09 (F)	Baseline	14.43	3.0	0.14	0	2	1	0
	Follow-up		2.0		0	2	1	0
P10 (M)	Baseline	9.64	1.0	0.21	0	1	3	0
	Follow-up		1.0		0	1	3	0
P11 (M)	Baseline	3.21	1.5	0.62	0	1	1	0
	Follow-up		2.0		0	0	1	0
P12 (M)	Baseline	0.76	2.0	1.32	2	6	7	5
	Follow-up		2.5		2	7	6	5
Mean \pm SD or median (IQR)	Baseline	11.0 \pm 8.1	2 (1.5–2.62)	0.25 (0.18–0.32)				
	Follow-up	N/A	2.25 (2–2.75)	N/A				

Abbreviations: +, positive; ARR, annualized relapse rate; EDSS, Expanded Disability Status Scale; F, female; IQR, interquartile range; M, male; QSM, quantitative susceptibility mapping; SD, standard deviation.

TABLE 2 MRI variables

MRI variables	Baseline	1-year follow-up	p value
Brain volume, cm ³			
MS	1516 (1489–1556)	1521 (1482–1540)	0.2
HC	1569 (1546–1611)		
p value (MS vs. HC)	0.010 [†]		
Thalamus volume, cm ³			
MS	14.4 (13.7–15.2)	14.3 (13.9–15.0)	0.13
HC	16.1 (15.5–16.8)		
p value (MS vs. HC)	0.010 [†]		
T2 lesion volume, cm ³	2.29 (1.18–3.27)	2.33 (1.67–3.25)	0.092

Note: Values are median (IQR).

Abbreviations: HC, healthy control; IQR, interquartile range; MRI, magnetic resonance imaging; MS, patient with multiple sclerosis.

[†]Statistically significant.

Clinical and conventional MRI parameters at baseline and follow-up amongst patients with MS

The median and interquartile range (IQR) EDSS score was 2 (1.5–2.62) at baseline and 2.25 (2–2.75) at follow-up. There were no serious adverse events, and no subjects suffered documented MS relapses. Focal T2 lesion burden was modest with a median (IQR) of 2.3 (1.2–3.3) cm³ T2 lesion volume per patient, and no significant alteration was observed in the lesion volume during follow-up (Table 2). At the individual lesion level, the median (IQR) volumes were 32 (17–101) mm³ and 34 (18–100) mm³ at baseline and follow-up, respectively, and no significant change in the number of T2 lesions of >8 voxels was detected with a median (IQR) of 19.5 (13.5–24.5) and 18.5 (14.0–25.5) at baseline and follow-up, respectively ($p > 0.05$). Whole brain and thalamic volumes were somewhat smaller amongst patients compared with controls, with no significant change during follow-up in either (Table 2).

Translocator protein PET binding amongst patients versus controls

Comparing TSPO binding between patients with MS and healthy controls, no baseline difference in TSPO availability (measured as DVR) was observed in any examined region (whole brain, NAWM, and thalamus) (Figure 1a–c). The mean (\pm SD) baseline DVRs of [¹¹C] PK11195 were 1.17 ± 0.03 and 1.17 ± 0.03 ($p = 0.84$) in the NAWM and 1.31 ± 0.05 and 1.28 ± 0.06 ($p = 0.27$) in the thalamus for patients versus controls, respectively. The presence of active voxels, that is, voxels with $\text{DVR} > 1.56$, was then evaluated and it was found that patients with MS had a greater baseline proportion of active voxels compared with controls ($7.7\% \pm 2.9\%$ vs. $5.4\% \pm 1.7\%$, $p = 0.033$), indicating modest innate immune cell activation (Figure 1d–f). When studied separately in the thalamus and NAWM, trends in the proportions of active voxels were similar but not statistically different (thalamus, $21.1\% \pm 5.5\%$ vs. $16.0\% \pm 7.3\%$, $p = 0.14$; NAWM, $7.0\% \pm 2.5\%$ vs. $5.2\% \pm 2.0\%$, $p = 0.052$, respectively).

Longitudinal stability of TSPO availability during teriflunomide treatment

The low level of baseline microglial activation remained stable during follow-up: whilst there was a slight trend downwards, there were no statistically significant DVR changes in the NAWM, thalamus or perilesional area between baseline and follow-up (Figure 2a–d). In the perilesional area, the baseline and follow-up DVRs were 1.15 ± 0.08 and 1.11 ± 0.08 ($p = 0.13$). In line with the DVR PET results, the proportion of active voxels remained stable during teriflunomide treatment in all examined brain areas (Figure 2e–h).

Prevalence of chronic active lesions at baseline based on TSPO-PET and QSM-MRI

Thirteen chronic lesions with iron rims were observed amongst six of 12 patients with MS at baseline and this number remained stable at follow-up. Similarly, according to our previously described TSPO-PET-based classification [33], six patients with MS had chronic rim active lesions at follow-up. Although the QSM-positive lesions did not reliably co-localize with PET-based rim activity, all patients with QSM-positive lesions had rim active lesions at follow-up, whilst none of the QSM-negative patients had rim active lesions at follow-up (Table 1). There were 35 chronic inactive lesions at baseline, with all patients having chronic inactive lesions. The number of overall active lesions was 27, with an average of 2.2 lesions per patient. All patients had overall active lesions either at baseline or at follow-up (Table 1). The majority (eight of 13) of QSM+ lesions were classified as overall active in PET at baseline.

DISCUSSION

Translocator protein availability remained largely unaltered during the 1-year follow-up in this cohort of teriflunomide-treated patients

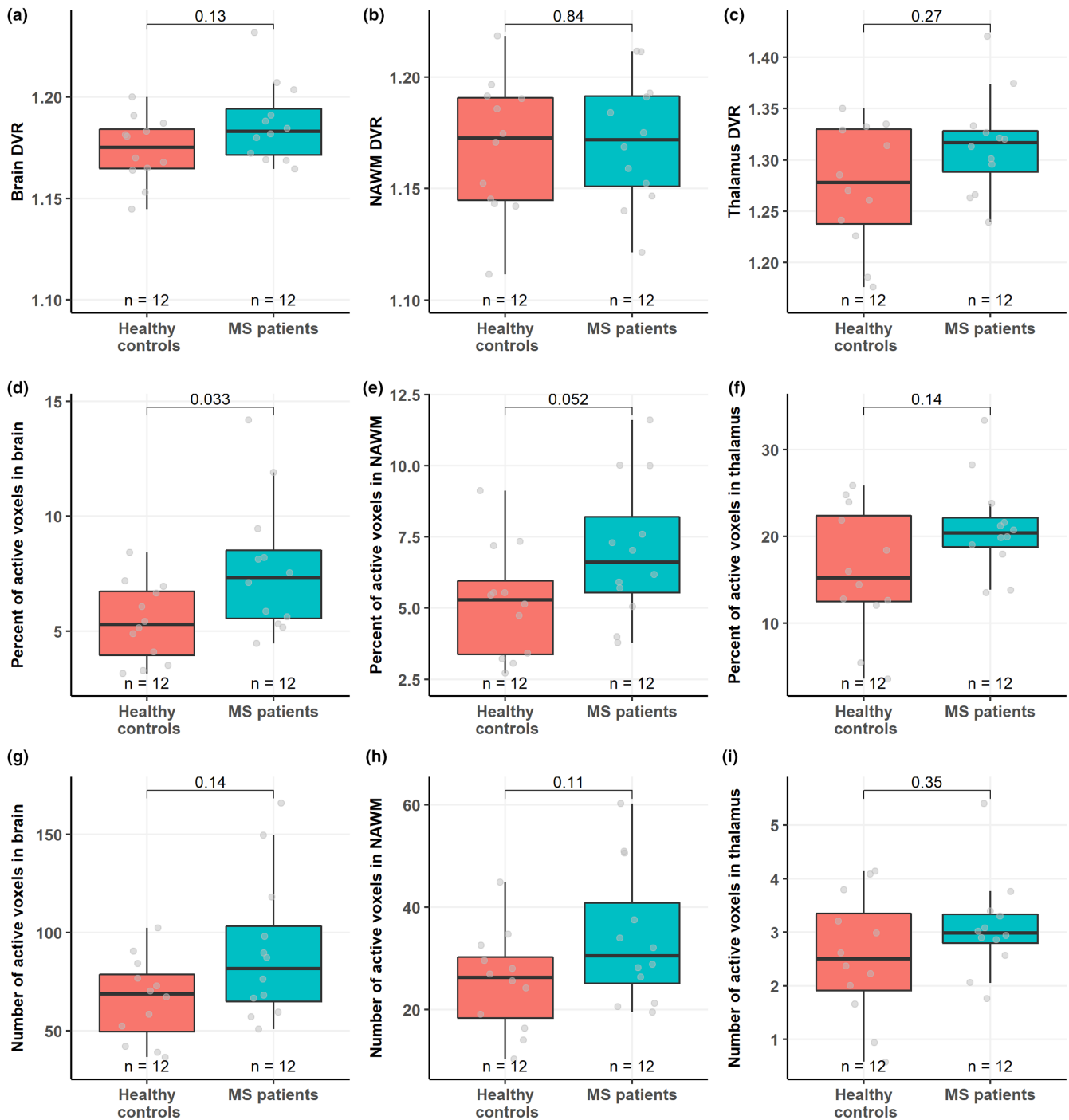


FIGURE 1 [^{11}C]PK11195 distribution volume ratio (DVR) comparisons in the whole brain (a), normal-appearing white matter (NAWM) (b) and thalamus (c) demonstrated no difference in 18-kDa translocator protein (TSPO) availability between patients with multiple sclerosis (MS) and healthy controls at baseline. The proportions (d)–(f) and absolute numbers (g)–(i) of active voxels were consistent with the DVR results.

with RRMS. Furthermore, only a modest difference in brain microglial activation was observed between the RRMS population and an age-matched healthy control population. However, this difference manifested as a statistically significant difference in the proportion of active voxels in the MS versus control brain. No difference in the DVR values in the whole brain, NAWM or thalamus regions of interest was observed between patients and controls. This finding

implies that TSPO-PET-based detection of clusters of active voxels in the MS brain may be a more sensitive method for capturing innate immune cell activation compared with quantifying radioligand binding using DVR in various brain regions of interest.

In other MS cohorts, significantly higher DVR values in MS brain NAWM compared to control white matter [13, 15, 16] have been previously repeatedly demonstrated by ourselves and others. It has

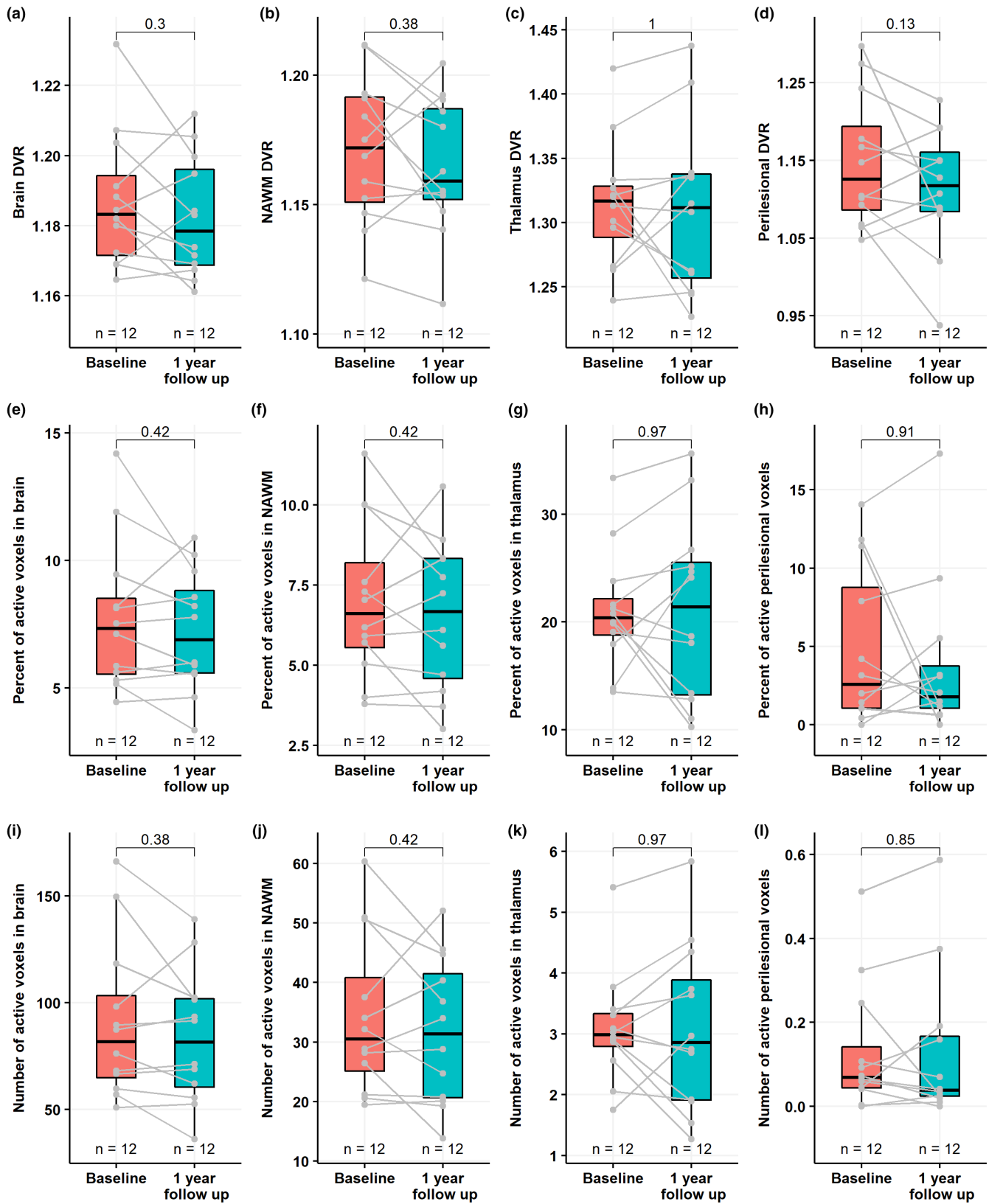


FIGURE 2 [¹¹C]PK11195 distribution volume ratio (DVR) comparisons in the whole brain (a), normal-appearing white matter (NAWM) (b), thalamus (c) and the perilesional area (d) demonstrated no alteration in 18-kDa translocator protein (TSPO) availability during 1-year treatment with teriflunomide in these regions of interest. Similarly, no significant differences were seen in the proportions (e)–(h) or absolute numbers (i)–(l) of active voxels.

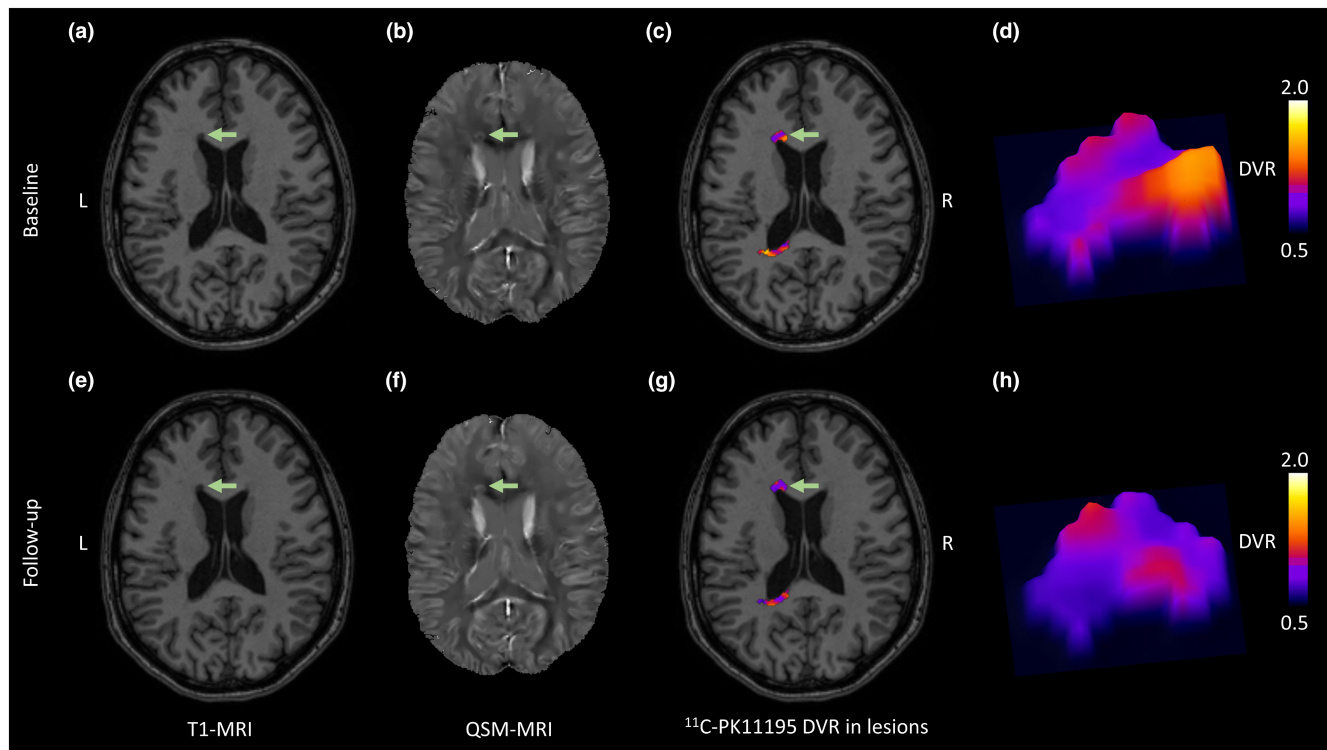


FIGURE 3 An example of an overall active lesion (P03) in T1 (a), quantitative susceptibility mapping (QSM) (b) and positron emission tomography (PET) overlaid on T1 (c) at baseline (a)–(c) and 1-year follow-up (e)–(g). The 3d surface plots (d), (h) represent PET distribution volume ratio (DVR) at baseline and follow-up, respectively, and the arrows point to the examined lesion. Whilst this lesion is not rim active according to our classification, a higher QSM intensity appears to co-localize with higher PET DVR at the lesion edge. L, left; R, right.

been demonstrated previously that higher TSPO-DVR both in the NAWM and thalamus [14, 34] predicts a greater likelihood of later disease progression, which is an indicator of the detrimental nature of TSPO-PET-measurable innate immune cell activation. Moreover, this finding may have implications for patient recruitment for future clinical trials targeting innate immune cell activation and slowing down disease progression or for clinical treatment stratification.

During the last decade, MS treatment has shifted towards early escalation and early intense treatment approaches [35]. This shift has happened at the expense of recent phase III trials [36, 37], where relatively less active cohorts of patients were enrolled compared with past trials [18, 19]. Although the more recent trials successfully demonstrated relative efficacy with limited effect size in RRMS in terms of the annualized relapse rate, prerequisites for successful trials on patients with SPMS should include concentrated efforts to better characterize the population at a high risk of progression.

Although conversion to secondary progression occurs on average at 45 years of age [38], MS is a heterogeneous disease, and, despite the relatively advanced age (46 years) and long disease duration (11 years) of the RRMS cohort included in the present study, only modest signs of TSPO-PET-measurable innate immune cell activation were visible, indicating a lower risk for disease progression in the near future. Additionally, the relatively high proportion of inactive lesions (>50% at baseline) and the low number of rim active lesions (six at baseline) suggest a lower risk of progression. A larger RRMS cohort ($n=67$) of comparable EDSS (median 2.5), with a

dissimilar distribution of inactive (34%) and rim active (16%) lesions, has been previously characterized, and it has been shown that rim active lesion load correlates with disability [33].

It is difficult to estimate exactly what impact teriflunomide treatment had on the longitudinally stable TSPO-PET findings in this cohort. It has been previously demonstrated in an untreated MS cohort with more accrued disability (EDSS=6) that TSPO binding increased over 1-year follow-up both in the NAWM and perilesional areas [15].

Teriflunomide limits the activation and proliferation of lymphocytes by inhibiting pyrimidine biosynthesis in activated lymphocytes via the mitochondrial enzyme dihydroorotate dehydrogenase [39]. Further to these peripheral effects, effects on resident CNS cells (oligodendrocytes and microglia) have been suggested based on in vitro and experimental animal work [40–42]. In rodents, teriflunomide crosses the blood–brain barrier with 1%–2% of serum concentrations (in the range 2.5–4.1 μM) reaching the CNS [43]. Thus, in theory, teriflunomide may have contributed to the unaltered microglial status via either peripheral or central effects.

From a health economic perspective, the correct timing of de-escalation or complete cessation of treatment is another unresolved question. Whilst a prior stable disease course does not appear to protect patients from disability after DMT discontinuation [44], discontinuation after a period of inactivity according to conventional clinical parameters was not associated with time to clinical relapse, MRI activity or EDSS increase [45]. The timing of discontinuation is not feasible with PET but based on the presented results and

previous evidence PET may be used to better characterize patients at a low risk of progression.

Other potential and more readily available prognostic biomarkers in MS include cortical MRI lesions [46], QSM [6], thalamic volume [47] and neurofilament light chain [48]. Future next steps include cross-sectional comparisons of these biomarkers with PET. Regarding our study cohort, follow-up including conventional MRI, diffusion tensor imaging, QSM and blood neurofilament light chain is ongoing up to 3 years after inclusion. In this study, only 13 QSM lesions were found, and the relatively short follow-up precluded meaningful correlations with clinical variables. However, QSM positivity seemed to correlate with microglial activity according to visual inspection of parametric PET data (Figures 3 and S1: between-group PET DVRs). Larger longitudinal studies that combine PET and QSM and also enroll patients with more active disease will demonstrate whether QSM is a more readily available surrogate for chronic smoldering inflammation and a possible temporal relationship between QSM intensity and later PET-measurable smoldering rim activity.

Our initial aspiration was to include a cohort that, based on patient age, was at risk of progression. However, as the study was performed in a real-life setting and age is a strong predictor of progression [20], the final study cohort was affected by relatively benign disease based on T2 lesion load (average 2.3 ml), low TSPO-PET DVR (not different from age-matched controls) and a low EDSS (median 2). Commonly employed SPMS criteria [49] require an EDSS of 4. Future trials targeting a population at progression risk would probably benefit from a higher EDSS requirement, although this would complicate recruitment efforts in the current treatment landscape, where active patients are often quickly identified and escalated, if necessary. Ultimately, even if combined, EDSS and age are unlikely to produce the required specificity or sensitivity.

Based on current knowledge, microglial activation contributes to MS progression and, beyond teriflunomide, novel compounds like Bruton's tyrosine kinase inhibitors currently in clinical development for MS may have greater potential to slow progression by suppressing activated microglia. The results of this study are in line with previous indirect evidence of teriflunomide's neuroprotective properties independent of relapse or conventional MRI activity [50]. Furthermore, a direct effect on brain microglia as a contributing mechanism of action cannot be excluded [40].

AUTHOR CONTRIBUTIONS

Marjo Nylund: Conceptualization; project administration; funding acquisition. **Markus Matilainen:** Formal analysis; visualization. **Marcus Sucksdorff:** Investigation; conceptualization. **Anna Vuorimaa:** Investigation. **Johan Rajander:** Resources. **Saara Wahlroos:** Resources. **Parisa Hariri:** Investigation; formal analysis.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

Any anonymized data used in the preparation of this article will be made available on the request of a qualified investigator.

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SUPPORTING INFORMATION

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