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In vivo assessment of neuroinflammation in progressive multiple sclerosis: a proof of concept study with [¹⁸F]DPA714 PET

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Abstract

Background: Over the past decades, positron emission tomography (PET) imaging has become an increasingly useful research modality in the field of multiple sclerosis (MS) research, as PET can visualise molecular processes, such as neuroinflammation, in vivo. The second generation PET radioligand [¹⁸F]DPA714 binds with high affinity to the 18-kDa translocator-protein (TSPO), which is mainly expressed on activated microglia. The aim of this proof of concept study was to evaluate this in vivo marker of neuroinflammation in primary and secondary progressive MS.

Methods: All subjects were genotyped for the rs6971 polymorphism within the TSPO gene, and low-affinity binders were excluded from participation in this study. Eight patients with progressive MS and seven age and genetic binding status matched healthy controls underwent a 60 min dynamic PET scan using [¹⁸F]DPA714, including both continuous on-line and manual arterial blood sampling to obtain metabolite-corrected arterial plasma input functions.

Results: The optimal model for quantification of [¹⁸F]DPA714 kinetics was a reversible two-tissue compartment model with additional blood volume parameter. For genetic high-affinity binders, a clear increase in binding potential was observed in patients with MS compared with age-matched controls. For both high and medium affinity binders, a further increase in binding potential was observed in T2 white matter lesions compared with non-lesional white matter. Volume of distribution, however, did not differentiate patients from healthy controls, as the large non-displaceable compartment of [¹⁸F]DPA714 masks its relatively small specific signal.

Conclusion: The TSPO radioligand [¹⁸F]DPA714 can reliably identify increased focal and diffuse neuroinflammation in progressive MS when using plasma input-derived binding potential, but observed differences were predominantly visible in high-affinity binders.

Keywords: [¹⁸F]DPA714, Multiple sclerosis, Neuroinflammation, Positron emission tomography, TSPO

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Background

Conventional magnetic resonance imaging [1] has a central role in the diagnosis of multiple sclerosis (MS), based on its high sensitivity for identifying MS-specific demyelinating lesions in the brain and spinal cord [2]. In patients with a relapsing-remitting disease course, radiological monitoring is based on the development of new MS lesions on T2-weighted MRI and the presence of gadolinium-enhancing lesions on T1-weighted MRI, demonstrating disruption of the blood-brain barrier which is indicative of active neuroinflammation [3]. However, these focal lesions as seen on MRI cannot fully explain the neurological and cognitive deficits in patients with MS [4]. In addition, in both primary and secondary progressive MS, neuroinflammation usually is not characterised by focal active demyelinating lesions [5]. Animal model and human post-mortem studies have identified a more diffuse and low-grade neuroinflammation behind an intact blood-brain barrier, not depicted by conventional MRI [6, 7]. Consequently, there is a need for novel imaging techniques.

Over the past decades, positron emission tomography (PET) imaging has become an increasingly useful research modality in the field of MS research, as PET can visualise molecular processes, such as neuroinflammation, in vivo [8, 9]. Specifically, the 18-kDa translocator-protein (TSPO) receptor, which is present on the mitochondrial membrane of microglial cells, with additional binding sites in monocytes, astrocytes and vascular endothelium, has shown potential as a target for imaging neuroinflammation with PET [10]. Using the first-generation TSPO PET tracer (*R*)-[¹¹C]PK11195, increased TSPO expression has been demonstrated in both MS lesions and normal appearing brain tissue of patients with MS, which was associated with increased disability and disease progression in all phases of the disease [11–16]. Unfortunately, (*R*)-[¹¹C]PK11195 suffers from poor brain penetration and high nonspecific binding in the brain, hindering accurate quantification [12]. Over the years, a large number of second-generation TSPO tracers have been developed, such as [¹¹C]PBR28, [¹⁸F]PBR111, [¹¹C]DPA713, [¹⁸F]GE180 and [¹⁸F]FEDAA1106. These novel tracers have an improved signal-to-noise ratio, resulting from lower lipophilicity and therefore less non-specific binding to e.g. plasma proteins and improved blood-brain barrier passage [17–21]. It has been shown that the second-generation radioligand [¹⁸F]DPA714 provides increased bioavailability to brain tissue and indeed has a higher signal-to-noise ratio [22, 23]. One limitation of second-generation tracers, including [¹⁸F]DPA714, is their genetically determined binding affinity, resulting from a single nuclide polymorphism rs6971 within the TSPO gene. Approximately 50% of the general population is a genetically determined high-affinity binder

(HAB), 30–40% a medium-affinity binder (MAB) and 10–20% a low-affinity binder (LAB) [24].

To date, quantification of cerebral [¹⁸F]DPA714 binding has been described in healthy controls [25] and in patients with Alzheimer's disease [26], with both studies agreeing on a reversible two-tissue compartment model with blood volume parameter ($2T4k_{VB}$) as the optimal kinetic model. In addition, pseudo-quantitative analyses have been performed in post-stroke [27] and Alzheimer's disease patients [28]. Moreover, the simplified reference tissue model, using cerebellar grey matter as a reference region, has been found to reliably assess binding potential in Alzheimer's disease [26]. However, the pathophysiology of MS violates the assumptions underlying reference tissue models: firstly, no single brain region can be used as a reference region in MS, as no region can be assumed to be devoid of specific TSPO binding, and secondly, the blood volume contribution is not negligible throughout the MS brain due to potential disruption of the blood-brain barrier [29]. The latter will also influence the kinetics of the tracer making reference tissue models unreliable [30].

The aim of this proof of concept study was to identify the optimal plasma input tracer kinetic model for characterising in vivo [¹⁸F]DPA714 kinetics in patients with primary and secondary progressive MS and to evaluate whether the kinetic parameters estimated from this model can be used to quantify TSPO binding in this patient group.

Methods

Participants

Patients diagnosed with primary or secondary progressive MS, according to the 2010 revisions of the McDonald criteria, and healthy controls were recruited from the MS Center, VU University Medical Center, Amsterdam, between January 2015 and February 2016. All subjects were genotyped for the rs6971 polymorphism within the TSPO gene, which predicts binding affinity to the second generation TSPO tracers [24]. Only genetically high and medium affinity binders (HAB and MAB) were included, and genetically low-affinity binders (LAB) were excluded. Further exclusion criteria for all subjects were a medical history of relevant neurological or auto-immune disease (other than MS); known cardiac, haematological, oncological or renal diseases; current or previous alcohol or drug abuse; recent treatment with immunomodulating drugs; or the use of benzodiazepines. None of the patients with progressive MS were on disease-modifying therapy, and no patients were treated with intravenous methylprednisolone for the last 3 months prior to the PET scans. All subjects had normal physical examination and all healthy controls a normal neurological examination.

This study was approved by the Medical Ethics Review Board of the VU University Medical Center and was registered under number 2014.356. All subjects gave written informed consent prior to participation.

MRI acquisition

MR imaging was performed on a 3 Tesla Ingenuity TF PET-MR system (Philips Medical Systems, Best, The Netherlands) on the same day as the PET-CT acquisition. MRI analysis included 3D T1 (repetition time 7.9 ms, echo time 4.5 ms, flip angle 8°, measured voxel size $1 \times 1 \times 1 \text{ mm}^3$) for region of interest (ROI) definition and 3D T2 Fluid Attenuated Inversion Recovery (FLAIR) (repetition time 4800 ms, echo time 279 ms, inversion time 1650 ms, measured voxel size $0.9 \times 0.9 \times 1.1 \text{ mm}^3$) for lesion segmentation. In patients, additional post-contrast 2D T1 sequences (repetition time 600 ms, echo time 10 ms, measured voxel size $0.56 \times 0.44 \times 5 \text{ mm}^3$) were acquired.

PET acquisition and reconstruction

Radiosynthesis of [^{18}F]DPA714 was performed with an in-house built automatic apparatus using procedures described previously [26]. PET scans were acquired on an Ingenuity TF PET-CT scanner (Philips Medical Systems, Best, The Netherlands). Following a low-dose CT scan for attenuation correction, a bolus of $263 \pm 13 \text{ MBq}$ [^{18}F]DPA714, with a mean molar activity of $39 \pm 20 \text{ GBq}/\mu\text{mol}$, was injected intravenously at 0.8 mL/sec with an automated infusion pump. Simultaneously, a 60-min PET dynamic emission scan was started. The emission scan was collected in list mode and rebinned into 19 frames (1×15 , 3×5 , 3×10 , 4×60 , 2×150 , 2×300 , $4 \times 600 \text{ s}$). PET data were reconstructed to a final voxel size of $2 \times 2 \times 2 \text{ mm}^3$ using standard scanner software (BLOB-OS-TF), which incorporates standard corrections for attenuation, scatter and randoms.

Arterial blood was withdrawn continuously from the radial artery during the entire scan (using an automated pump for the first 5 min at 5 mL/min and from 5 to 60 min at 2.5 mL/min), and in addition, manual samples were collected at six time points (5, 10, 20, 30, 40 and 60 min) [31]. Continuous whole blood time activity curves (TACs) were corrected for plasma to whole blood ratios, metabolites and time delay to obtain metabolite-corrected arterial plasma input functions.

Data analysis

MS white matter lesions were automatically segmented on the FLAIR and T1-weighted images using kNN-TTP [32]. Lesion filling of the T1-weighted image was obtained using LEAP [33], after which the filled T1-weighted image was registered to the PET scan using the VINCI software package [34, 35]. Grey matter, white matter and cerebral spinal fluid segmentation was performed automatically

using SPM8 implemented in PVElab [36]. ROIs were defined according to the Hammers template on the co-registered 3D T1 MRI [37]. Regional TACs were extracted by superimposing the MR-derived ROIs onto the dynamic PET images. Volume weighted larger ROIs were also defined to assess the impact of ROI size on quantitative analyses. The lesion masks were subtracted from segmented white matter to define non-lesional white matter.

Kinetic modelling was performed using standard single-tissue (1T2k) together with reversible and irreversible two-tissue (2T3k and 2T4k) compartment models, both with and without blood volume parameter (V_B) [38]. It has been hypothesised that some binding to the vascular endothelium exists for TSPO tracers, which can be characterised by slow irreversible binding (K_b) [39, 40]. Therefore, a 2T4k V_B with an irreversible vascular binding component (1T1k) was also tested. Akaike information criterion (AIC) was used to compare model fits and to identify the optimal tracer kinetic model. Reliability of parameter estimates was determined by the percentage standard deviation (%SD) of these estimates, with a cut-off of 25% for the generally robust K_1 , and 50% for the other parameters. Estimates for K_1 , BP_{ND} (k_3/k_4), V_T and K_b from the optimal model were used to assess group differences between patients and controls, and between genetically high and medium affinity binders (HAB and MAB).

Statistical analysis

Group differences were evaluated using the Mann-Whitney U test in SPSS 22.0 (IBM Corp., Armonk, NY). Due to the explorative nature of this proof of concept study, we did not correct for multiple comparisons. To evaluate regional differences within the patient group, a Wilcoxon signed-rank test was used.

Results

Demographics

At screening, all subjects were genotyped for the rs6971 polymorphism within the TSPO gene and low-affinity binders were excluded from participation in this study. In total, eight patients diagnosed with primary or secondary progressive MS and seven healthy controls completed the whole study protocol. Demographic information is reported in Table 1. Patients included were four HAB and four MAB, with a mean age of 53 ± 3 years and a wide variation in T2 lesion load, ranging from a few solitary lesions to widespread confluent lesions (3.6 to 60.6 cm^2). In only two patients, one small enhancing lesion could be identified, not uncommon in primary or secondary progressive MS. In healthy controls, genetic binding status was similarly distributed: three HAB and four MAB. The mean age for healthy controls was 52 ± 4 years, which was not significantly different from the patients with MS ($p = 0.418$).

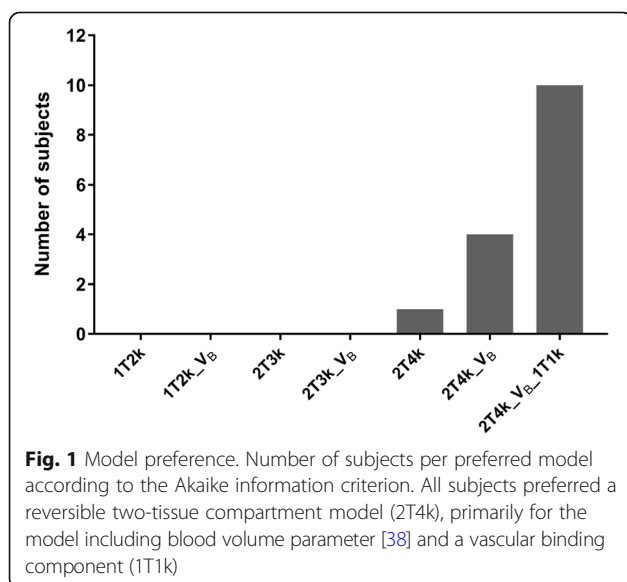
Table 1 Demographical characteristics

	Patients (n = 8)	Healthy controls (n = 7)
Age, mean and SD (years)	53.1 ± 2.7	52.0 ± 4.1
Gender, male/female	3/5	3/4
Genotype, HAB/MAB	4/4	3/4
Subtype MS, PPMS/SPMS	5/3	N/A
Disease duration, mean and SD (years)	13.1 ± 9.4	N/A
EDSS, median and range	5.0 (4.0–6.0)	N/A
PASAT-3 score, mean and SD	45.8 ± 10.8	49.6 ± 12.5
SDMT score, mean and SD	44.0 ± 11.9	55.0 ± 13.5
T2 lesion volume, median and range (cm ³)	14.3 (3.6–60.6)	N/A

Abbreviations: EDSS Expanded Disability Status Scale, HAB genetic high-affinity binder, MAB genetic medium-affinity binder, PASAT Paced Auditory Serial Addition Test, PPMS primary progressive multiple sclerosis, SDMT Symbol Digit Modalities Test, SPMS secondary progressive multiple sclerosis

Tracer kinetics

Based on the AIC, for the majority of subjects, the preferred model for quantification of [¹⁸F]DPA714 was 2T4k_V_B_1T1k, see Fig. 1. However, when evaluating reliability of the parameter estimates, for a large number of regions, K₁, BP_{ND} and/or K_b could not be reliably estimated, with either a high %SD or values touching the boundaries (see Additional files 1 and 2). This was even the case for subjects in which 2T4k_V_B_1T1k was the preferred model according to AIC, and it was true not only for small, but also for large ROIs. As model fits for 2T4k_V_B_1T1k and 2T4k_V_B were comparable (see Fig. 2) and 2T4k_V_B did result in reliable parameter estimates, the latter model was used for further analyses.



Volume of distribution

Mean regional V_T values were on average 1.6 times higher in HABs than in MABs (Fig. 3a). There was no clear regional difference in V_T between patients and healthy controls for both HABs and MABs, as Fig. 3a illustrates for the MS white matter lesions, non-lesional white matter and whole brain grey matter. Moreover, very limited within-subject variation in V_T was observed, even for the patients with MS. Figure 3b illustrates that all subjects showed a similar distribution pattern for V_T throughout the brain. In addition, a strong correlation between V_T and K_1 was seen (Fig. 4a), whereas no correlations were found between V_T and both k_2 and k_3/k_4 .

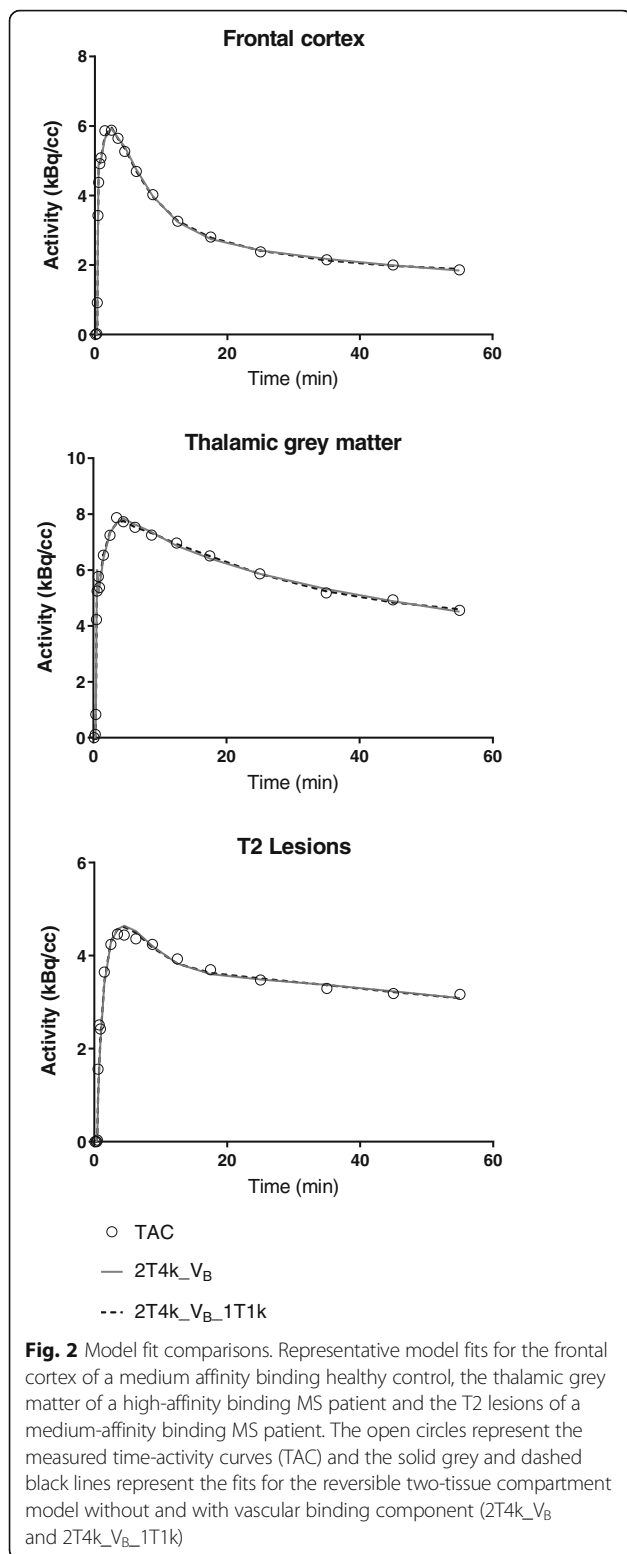
Finally, regional V_T values for the 2T4k_V_B_1T1k model were evaluated (Fig. 3c). As expected, the added vascular binding component decreased the V_T . As the regional V_T values for the two models were strongly correlated for the large ROIs, there was also no difference in 2T4k_V_B_1T1k V_T between patients and healthy controls for both the HABs and MABs (Fig. 3c). It is of interest to know that also the correlation between V_T and K_1 as seen for the simpler model remained the same for this model incorporating a vascular binding component (Fig. 4b).

Binding potential

BP_{ND} (k_3/k_4) estimates for the larger ROIs were reliable using the 2T4k_V_B model, as for almost all regions, the standard deviation was well below 50% for both HABs and MABs (in both patients and controls), see Additional file 3. This indicates that plasma input-derived BP_{ND} can be used as outcome parameter for these larger brain regions. Like V_T , regional BP_{ND} was on average 1.6 times higher in HAB than in MAB subjects (Fig. 5). For the regions shown in Fig. 5, this difference was statistically significant for frontal cortex ($p = 0.001$), cingulate cortex ($p = 0.006$), thalamic grey matter ($p = 0.002$) and brainstem white matter ($p = 0.004$). In contrast to the V_T findings, regional BP_{ND} in both grey and white matter of HAB patients was higher than in HAB healthy controls. This difference was statistically significant for cingulate cortex ($p = 0.034$), cerebellar white matter ($p = 0.034$) and non-lesional white matter ($p = 0.034$). This difference was not observed in the MAB group.

Multiple sclerosis lesions

In both HAB and MAB patients with MS, MRI-defined T2 white matter lesions showed a significant increase in BP_{ND} compared with non-lesional white matter of the same subject ($p = 0.017$) (Fig. 6a). There was a strong correlation between the BP_{ND} values, except for one patient. When including all the subjects, the correlation coefficient (R^2) was 0.246 (slope 1.04 ± 0.744), excluding



the one outlier improved the R^2 to 0.861 (slope 2.39 ± 0.429). For V_T , values were similar for lesions and non-lesional white matter of each MS patient, with an R^2 of 0.844 and slope 1.02 ± 0.178 (Fig. 6b).

Discussion

This proof of concept study in patients with progressive MS showed that quantitative assessment of the second-generation TSPO radiotracer $[^{18}\text{F}]\text{DPA714}$ can identify an increased binding potential not only in MS-specific lesions, but for HAB patients also in grey matter and non-lesional white matter. Although the results from this study need to be replicated in a larger cohort, they suggest that $[^{18}\text{F}]\text{DPA714}$ -PET could be used as an in vivo imaging technique for measuring neuroinflammation in progressive MS.

Neuroinflammation in MS is characterised by complex and dynamic processes, including the activation of microglia [6, 7]. This is most distinct in active MS lesions, identified by gadolinium enhancement on T1-weighted MRI, and chronic MS lesions as seen on T2-weighted MRI [3]. In line with this, an increase in $[^{18}\text{F}]\text{DPA714}$ BP_{ND} was seen in the focal lesions compared with non-lesional white matter for all patients except one, independent of the patient's genetic binding status (HAB or MAB). As MRI-defined lesions for these patients with progressive MS were almost exclusively non-enhancing chronic lesions, this increase TSPO binding indicates that these are chronic active lesions [41]. The classification of different stages of MS lesions, such as chronic active and chronic inactive lesions, results from pathological studies. Current conventional MRI techniques can identify active inflammatory lesions, with the use of gadolinium-enhanced T1-weighted scans; however, they cannot further differentiate non-enhancing lesions. Results from the present study suggest that $[^{18}\text{F}]\text{DPA714}$ PET can identify different stages of MS lesions in vivo, which could be clinically relevant, e.g. in relation to treatment options and monitoring.

Even more importantly, increased $[^{18}\text{F}]\text{DPA714}$ BP_{ND} was also seen in non-lesional brain parenchyma, both in white and grey matter, for HAB patients. In relapsing-remitting MS, neuroinflammation is evident from the development of new focal (gadolinium enhancing) lesions, but in primary and secondary progressive MS, neuroinflammation is often more diffuse and low-grade [5]. Therefore, it is highly relevant that a biomarker of neuroinflammation in MS demonstrates not only focal changes, but also more diffuse pathology. As $[^{18}\text{F}]\text{DPA714}$ BP_{ND} can identify both, this marker of TSPO expression can be used as a biomarker to further investigate neuroinflammation in MS in vivo.

As plasma input-derived BP_{ND} was estimated reliably using the 2T4k_VB model, BP_{ND} should be preferred over the less specific V_T . As V_T contains both free, non-specific and specific signals, it is an inferior outcome measured compared with BP_{ND} ($=k_3/k_4$), which only reflects specific binding. Contrary to BP_{ND} analysis, group analysis using V_T showed no difference between patients and controls for both HAB and MAB subjects.

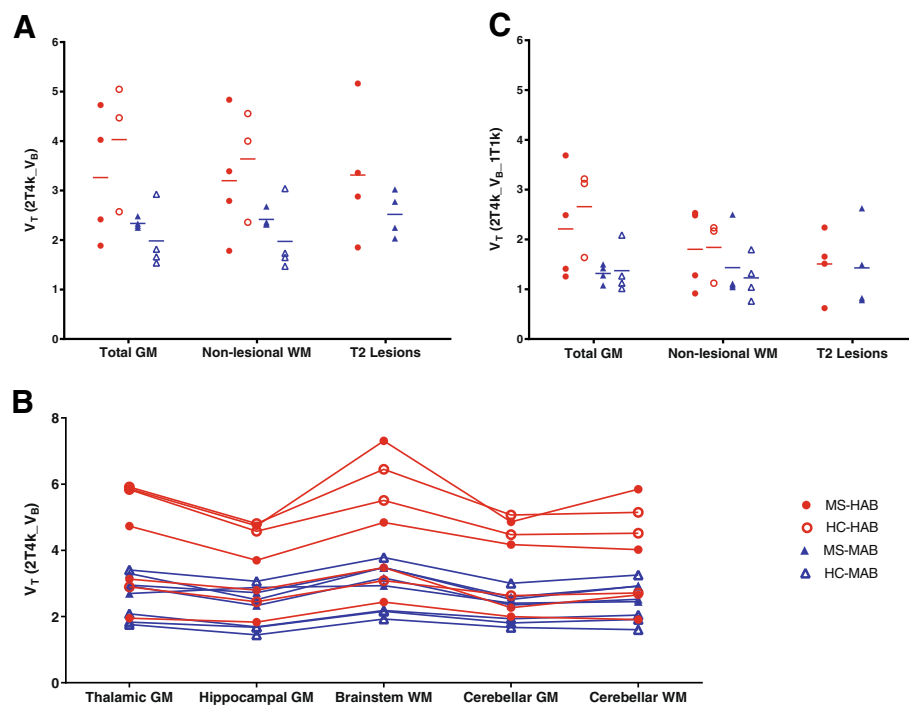


Fig. 3 Volume of distribution. **a** Volume of distribution (V_T) for total grey matter non-lesional white matter (WM) and T2 defined white matter lesions for the reversible two-tissue compartment model (2T4k_ V_B). High-affinity binding (HAB) subjects in red have a 1.5 to 2-fold higher V_T than medium affinity binding (MAB) subjects in blue. No difference in V_T was seen between multiple sclerosis patients (MS; closed symbols) and healthy controls (HC; open symbols) for both HABs and MABs. **b** V_T across various regions of all patients and controls. The intra-subject variation appears to be much smaller than the inter-subject variation. **c** The reversible two-tissue compartment model with vascular binding component (2T4k_ V_B _1T1k) also identified an increase in V_T for HAB subjects compared with MAB subjects, but no difference in mean regional V_T for patients compared to controls for either HABs or MABs

In addition, V_T appeared to be almost similar between different ROIs within each subject, resulting from a nearly homogeneous distribution of DPA714 throughout the brain of an individual. As V_T includes the large non-displaceable compartment of [^{18}F]DPA714, this effectively obscured any changes in the disease-specific neuroinflammatory signal. This is also seen in the analysis of the T2 MS lesions in Fig. 6, illustrating that the increase in specific [^{18}F]DPA714 signal in MS lesions depicted by BP_{ND} is diluted in the V_T values due to the non-displaceable component.

Interestingly, V_T was strongly correlated with K_1 . Although a change in K_1 can be related to neuroinflammation, as K_1 is a function of blood flow and vascular permeability, a correlation between V_T and K_1 was unexpected as V_T should be independent of K_1 . A possible explanation for this finding could be related to binding of [^{18}F]DPA714 to the endothelium of blood vessels in the brain [42, 43]. However, the model incorporating a vascular binding component still provided correlated K_1 and V_T values (Fig. 4b).

Although the 2T4k_ V_B _1T1k model was preferred according to AIC, it decreased the reliability of the parameter estimates compared with the simpler 2T4k_ V_B

model. Moreover, K_b estimates were inaccurate for many regions, even in subjects where this model was preferred. A proposed method of decreasing the number of parameters, attempting to increase their reliability, is fixing V_B to 5%. However, due to the large inter- and intra-subject variation in V_B , this seems inappropriate.

Although we used the simpler model in our analysis, the results are similar, as the regional V_T values for the two models correlate.

In line with other TSPO studies, assessment of the rs6971 polymorphism defined binding status is important also for quantitative analysis of [^{18}F]DPA714. Although observed differences between HABs and MABs were smaller than those observed for some other second-generation TSPO tracers [24], both mean V_T and BP_{ND} values were on average 1.6 times higher for HABs compared with MABs for the different brain regions. This indicates that the selection of subjects based on binding status is essential. Furthermore, group differences between HABs were only observed when using BP_{ND} , suggesting that [^{18}F]DPA714 in MS could best be studied in HABs only. Due to basal expression of TSPO, the contrast for [^{18}F]DPA714 appears to be too low for MAB subjects to identify patients from controls. Strikingly, the increase in

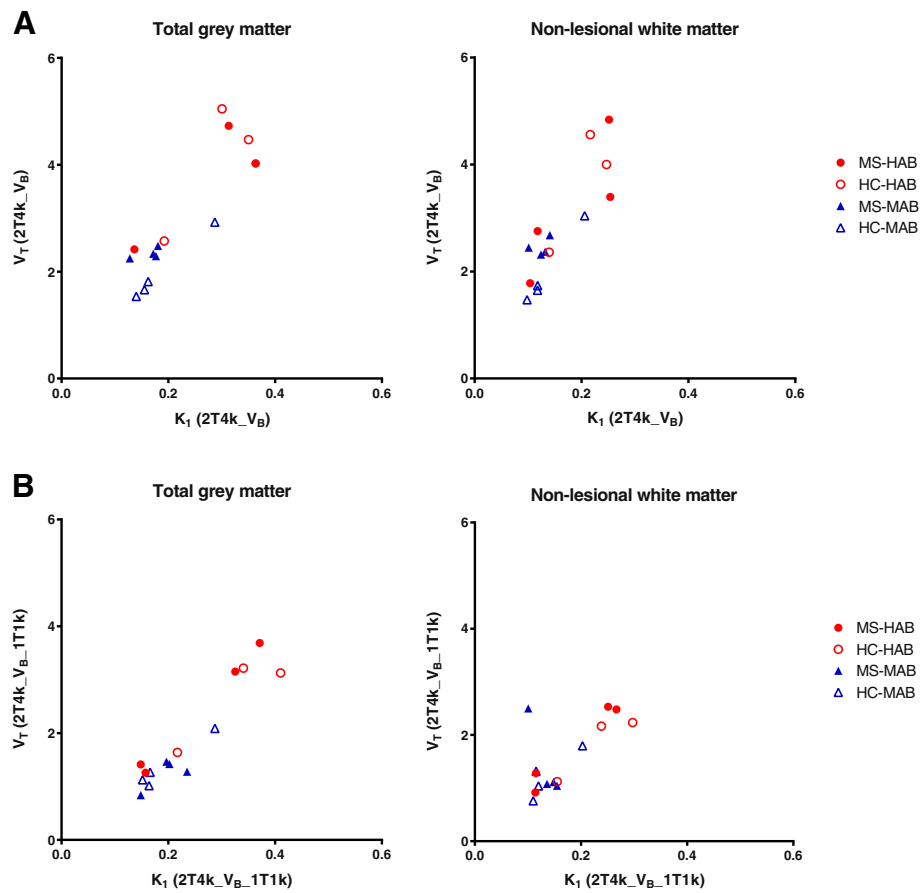


Fig. 4 Correlation for V_T and K_1 . For both the reversible two-tissue compartment model **a** without and **b** with vascular binding component ($2T4k_V_B$ and $2T4k_V_B_1T1k$), there was a strong correlation between V_T and K_1 in both grey and white matter, independent of binding status

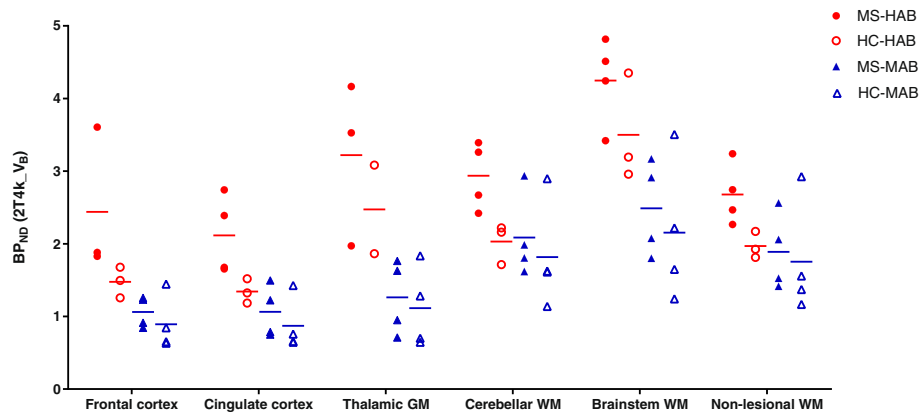
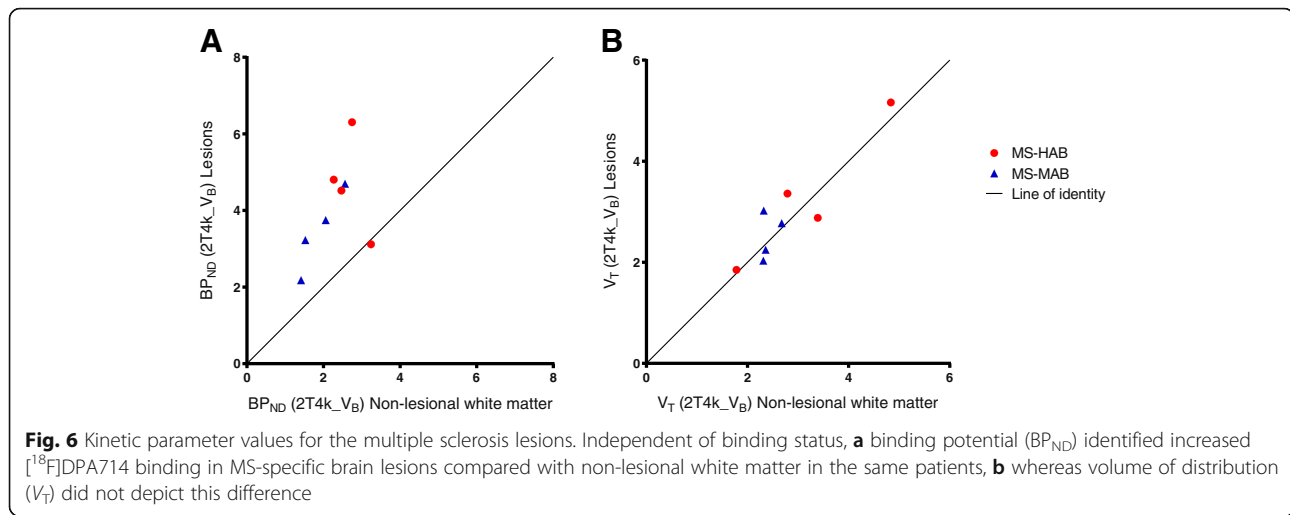


Fig. 5 Binding potential for different regions of interest. For high-affinity binders, a higher binding potential (BP_{ND}) was seen in multiple sclerosis patients (MS-HAB) than in healthy controls (HC-HAB) for different regions of interest. This difference was not seen in medium-affinity binding (MAB) subjects



BP_{ND} in MS lesions was also seen in MAB patients, indicating that [^{18}F]DPA714 can identify neuroinflammation in MAB subjects in MS lesions, but due to high non-specific binding of the tracer, it is not evident in other ROIs with more low-grade neuroinflammation. As only approximately half of the general population is HAB, this is a limitation of second-generation TSPO studies.

This study is limited mainly by its small sample size. Because of the limited number of subjects in each group, only limited statistical analysis was performed. Nevertheless, based on the promising results of this pilot project, further studies using a larger sample size are warranted. This would allow for evaluation of possible correlations between [^{18}F]DPA714 BP_{ND} and clinical outcome scores, such as Expanded Disability Status Scale and Symbol Digit Modalities Test. Finally, assessment of a possible relationship with MRI-derived outcome measures, such as atrophy of cortical and deep grey matter structures, would be of great interest in understanding progressive MS.

Conclusions

Plasma input-derived BP_{ND} can reliably be estimated for [^{18}F]DPA714, and a 2T4k- V_B model [^{18}F]DPA714 BP_{ND} can identify increased neuroinflammation in both grey and non-lesional white matter of patients with progressive MS, with a further increase in T2 MS lesions. As these differences cannot be seen using V_T due to inclusion of non-displaceable uptake, BP_{ND} is recommended for analysis of [^{18}F]DPA714 studies in patients with MS. Similar to other second-generation TSPO tracers, the effect of genetically defined binding status limits the clinical use of [^{18}F]DPA714. Nevertheless, further studies are warranted to confirm the results of the present proof of concept study.

Additional files

Additional file 1: 2T4k- V_B -1T1k - Percentage standard deviation for BP_{ND} (k_3/k_4). Additional file provides the percentage of standard deviation for the for 2T4k- V_B -1T1k BP_{ND} (k_3/k_4) for the different large regions of interest. (PDF 106 kb)

Additional file 2: 2T4k- V_B -1T1k - Percentage standard deviation for k_b . Additional file provides the percentage of standard deviation for the for 2T4k- V_B -1T1k k_b for the different large regions of interest. (PDF 106 kb)

Additional file 3: 2T4k- V_B - Percentage standard deviation for BP_{ND} (k_3/k_4). Additional file provides the percentage of standard deviation for the arterial input-derived BP_{ND} (k_3/k_4) for the different large regions of interest. (PDF 106 kb)

Abbreviations

1T2k: Single-tissue compartment model; 2T3k: Irreversible two-tissue compartment model; 2T4k: Reversible two-tissue compartment model; BP_{ND} : Binding potential (k_3/k_4); FLAIR: Fluid Attenuated Inversion Recovery; HAB: High-affinity binder; k_b : Vascular binding component; LAB: Low-affinity binder; MAB: Medium-affinity binder; MRI: Magnetic resonance imaging; MS: Multiple sclerosis; PET: Positron emission tomography; ROI: Region of interest; TAC: Time activity curve; TSPO: 18-kDa translocator-protein; V_B : Blood volume parameter; V_T : Volume of distribution

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

The study was designed by MH, AMVD, JK, ADW, FB and BvB. Tracer development was done by TAR, MK and ADW. Data were collected by MH, MTW, PS and JB. Analysis of the data was performed by MH, SVG, MY, DH, MDS, RCS and AAL and data were interpreted by MH, SVG, MY, BvB and AAL. The manuscript was drafted by MH and SVG. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Medical Ethics Review Board of the VU University Medical Center and was registered under the number 2014.356. All subjects gave written informed consent prior to participation.

Consent for publication

Not applicable.

Competing interests

DH is employed by Philips Healthcare. JK has accepted speaker and consultancy fees from Merck-Serono, Teva, Biogen, Genzyme, Roche and Novartis. FB is supported by the NIHR UCLH Biomedical Research Centre and serves as a consultant for Bayer-Schering Pharma, Sanofi-Aventis, Genzyme, Biogen-Idec, Teva, Novartis, Roche, Synthron BV and Jansen Research. All other authors declare that they have no competing interests.

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