Reliable quantification of $^{18}$F-GE-180 PET neuroinflammation studies using an individually scaled population-based input function or late tissue-to-blood ratio

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Received: 20 December 2019 / Accepted: 2 April 2020 / Published online: 23 April 2020 © The Author(s) 2020

Abstract

**Purpose** Tracer kinetic modeling of tissue time activity curves and the individual input function based on arterial blood sampling and metabolite correction is the gold standard for quantitative characterization of microglia activation by PET with the translocator protein (TSPO) ligand $^{18}$F-GE-180. This study tested simplified methods for quantification of $^{18}$F-GE-180 PET.

**Methods** Dynamic $^{18}$F-GE-180 PET with arterial blood sampling and metabolite correction was performed in five healthy volunteers and 20 liver-transplanted patients. Population-based input function templates were generated by averaging individual input functions normalized to the total area under the input function using a leave-one-out approach. Individual population-based input functions were obtained by scaling the input function template with the individual parent activity concentration of $^{18}$F-GE-180 in arterial plasma in a blood sample drawn at 27.5 min or by the individual administered tracer activity, respectively. The total $^{18}$F-GE-180 distribution volume ($V_T$) was estimated in 12 regions-of-interest (ROIs) by the invasive Logan plot using the measured or the population-based input functions. Late ROI-to-whole-blood and ROI-to-cerebellum ratio were also computed.

**Results** Correlation with the reference $V_T$ (with individually measured input function) was very high for $V_T$ with the population-based input function scaled with the blood sample and for the ROI-to-whole-blood ratio (Pearson correlation coefficient = 0.989 ± 0.006 and 0.970 ± 0.005). The correlation was only moderate for $V_T$ with the population-based input function scaled with tracer activity dose and for the ROI-to-cerebellum ratio (0.653 ± 0.074 and 0.384 ± 0.177). Reference $V_T$, population-based $V_T$ with scaling by the blood sample, and ROI-to-whole-blood ratio were sensitive to the TSPO gene polymorphism. Population-based $V_T$ with scaling to the administered tracer activity and the ROI-to-cerebellum ratio failed to detect a polymorphism effect.

**Conclusion** These results support the use of a population-based input function scaled with a single blood sample or the ROI-to-whole-blood ratio at a late time point for simplified quantitative analysis of $^{18}$F-GE-180 PET.

**Keywords** Translocator protein (TSPO) · Flutriciclamide · GE-180 · Kinetic analysis · Input function · Population-based

This article is part of the Topical Collection on Neurology

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00259-020-04810-1) contains supplementary material, which is available to authorized users.

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Introduction

There is increasing evidence that chronic neuroinflammation caused by cells of the innate neuroimmune system after activation by danger-associated molecular patterns such as misfolded proteins contributes to the pathogenesis of neurodegenerative diseases [1, 2]. Chronic pro-inflammatory reactions of the neuroimmune system most likely play a prominent role also in various other neurological and psychiatric diseases including stroke [3], multiple sclerosis [4], brain tumors [5], hepatic encephalopathy [6], and major depression [7].

Microglia is the major cell type of the neuroimmune system [1]. Its activation is associated with an increased expression of the translocator protein (TSPO), an 18-kDa, five transmembrane domain protein primarily located in the outer mitochondrial membrane and formerly known as peripheral benzodiazepine receptor [8–11]. Positron emission tomography (PET) imaging with radiolabeled TSPO ligands therefore is a promising modality for detection, quantitative characterization, and monitoring of neuroinflammation in vivo [12]. In patients with mild cognitive impairment or mild dementia of the Alzheimer type, for example, PET with the first-generation TSPO PET tracer [11C](R)-PK11195 [13] demonstrated increased tracer binding in the brain regions with the most prominent synaptic dysfunction/degeneration in Alzheimer’s disease, suggesting microglial activation at early clinical stages of the disease [14].

Limitations of [11C](R)-PK11195 include its rather low signal-to-background binding ratio in vivo [15] and the short 20 min physical half-life of the radioactive label. The latter restricts the use of [11C](R)-PK11195 to centers with cyclotron and radiochemistry on site. This prompted the development of novel TSPO PET ligands with improved pharmacokinetics and labeled with 18F (110 min half-life). Amongst these second- and third-generation TSPO PET ligands is the tricyclic indole compound (S)-N,N-diethyl-9-(2-[18F]fluoroethyl)-5-methoxy-2,3,4,9-tetrahydro-1H-carbazole-4-carboxamide (Flutriciclamide, 18F-GE-180) [16]. Superior pharmacokinetics (higher binding potential) compared to [11C](R)-PK11195 has been demonstrated in a lipopolysaccharide-induced rat model of acute neuroinflammation [17] and in a rat model of stroke [18]. 18F-GE-180 provided higher sensitivity to detect microglial activation and its changes under therapy in a mouse model of Alzheimer’s disease compared to the second-generation TSPO tracer 18F-PBR06 [19].

The standard method for quantitative characterization of 18F-GE-180 binding in humans is full tracer kinetic modeling of tissue time activity curves (TAC) measured by PET via acquisition of a sequence of image frames covering a total duration of at least 90 min starting with injection of 18F-GE-180 [20, 21]. The input function required for full kinetic modeling, that is, the time course of unmetabolized 18F-GE-180 in arterial plasma in brain capillaries, is derived from automatically and/or manually drawn arterial blood samples using high-performance liquid chromatography (HPLC) analysis to separate radioactive metabolites. Arterial blood sampling during the whole scan duration and HPLC processing of (≥ 6) discrete blood samples is not only burdensome for both patient and staff but it also restricts the use of 18F-GE-180 PET to centers with radiochemical facility.

Reference tissue methods that allow quantitative estimates of tracer binding by comparing the TAC in the region-of-interest (ROI) with the TAC in a reference tissue region free of the imaging target (TSPO) and, therefore, do not require the arterial input function, are widely used. Application of reference tissue methods in TSPO PET is limited by the lack of a brain region in which microglial activation can be ruled out a priori in all subjects under all conditions [20]. Supervised clustering of tissue TACs on the voxel level [22, 23], voxel-based statistical testing of a late standard uptake value (SUV) image of healthy controls versus a group of patients with the disease of interest [24], and crescent-shaped ROIs manually placed in brain regions with visually normal tracer uptake [25] have been proposed for the identification of an appropriate tissue reference region in TSPO PET. These methods have been proven useful for quantitative characterization of lesions with high microglial activation. They might be limited if there is no valid reference region due to possible widespread global neuroinflammation.

Population-based input functions for tracer kinetic modeling have been successfully used for quantitative analysis of brain PET with FDG [26–29] and a variety of other tracers [30–35]. The aim of the present study was to evaluate methods for quantitative analysis of 18F-GE-180 PET using population-based blood curves that either require only a single late blood sample or no blood at all. The tissue-to-whole-blood ratio at a late time point was also tested. The latter does not require dynamic PET imaging (only a late static uptake image) and has been shown to be an excellent surrogate of quantitative parameters from modeling of dynamic PET data for other tracers [36, 37].

Materials and methods

Subjects

The study included a total of 25 subjects (age 59.4 ± 9.4 years, range 37–77 years, 7 females), 20 patients after liver transplantation and 5 healthy subjects from an ongoing prospective study on the effect of immunosuppression on microglial activity in liver-transplanted patients. Nine of the 20 liver-transplanted patients were on standard dose immunosuppression with the calcineurin inhibitors (CNI) tacrolimus or ciclosporin with or without combination with other
immunosuppressants such as mycophenolat mofetil. Nine liver-transplanted patients were on reduced CNI dose. The remaining two liver-transplanted patients were on immunosuppressive therapy free of CNI. All patients after liver transplantation included in this study had restored liver function.

**DNA extraction and polymorphism genotyping**

The binding of most (if not all) TSPO ligands is affected by a single nucleotide polymorphism in the TSPO gene (SNP rs6971) leading to an Ala147Thr amino-acid substitution which affects affinity of the TSPO for binding of the PET ligands. This results in systematic inter-subject variability of TSPO ligand kinetics depending on the genotype (high-affinity binder, HAB, or low-affinity binder, LAB, or mixed affinity binder, MAB) [38].

Genotyping was performed as described previously [25]. In brief, genomic DNA was extracted from 4 mL of whole blood with QIAamp DNA blood maxi kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. DNA quality was assessed utilizing optical absorbance and gel electrophoresis. Exon 4 of TSPO gene, as well as exon/intron junctions, were PCR amplified and sequenced using the Sanger method with the following primers: ex4-F-AGTTGGGCAGTGGGACAG and ex4-R-GCAGATCC TGCAGAGACGA. Sequencing data were analyzed using SnapGene software (GSL Biotech; available at snapgene.com).

Fourteen subjects (11 patients/3 healthy control subjects) were HAB, 8 subjects (7/1) were MAB, and 3 subjects (2/1) were LAB (Table 1). HAB subjects were slightly older than MAB subjects, the difference just barely missed statistical significance (61.6 ± 9.3 versus 54.0 ± 9.0 years, two-sided t test \( p = 0.077 \)). HAB subjects and MAB subjects did not differ with respect to sex (36 versus 25% females, chi-square \( p = 0.604 \)).

**Synthesis of \(^{18}\text{F}-\text{GE}-180\)**

\(^{18}\text{F}-\text{GE}-180\) was produced in a GMP-compliant synthesis using a single use disposable cassette (FASTlab PET GE-180 cassette, GE Healthcare, UK) on an automated synthesizer system (FASTlab™, GE Healthcare, UK) and the corresponding S-enatiomeric pure mesylate precursor (3.5 mg, GMP grade) [39]. \(^{18}\text{F}\)fluoride was obtained from a 11-MeV cyclotron (Eclipse HP, Siemens, Knoxville, USA) using the \(^{18}\text{O}(\text{p},\text{n})^{18}\text{O}\) nuclear reaction on enriched (97–98%) \(^{18}\text{O}\)water, and directly transferred to the radiosynthesizer. After 45 min automated synthesis, the product \(^{18}\text{F}-\text{GE}-180\) was obtained as the pure \(\text{S}^-\text{enatiomer (}\text{S})\text{-N,N-diethyl-9-(2-[^{18}\text{F}]fluoroethyl)-5-methoxy-2,3,4,9-tetrahydro-1H-carbazole-4-carboxamide [40] in a sterile buffer solution (35–37 ml). The radiochemical yield was 42% ± 5% with radiochemical purity of ≥97% and specific activity of 517 ± 54 GBq/μmol. Quality control tests were performed according to GMP and the EU pharmacopoeia. All batches met the required acceptance criteria and were released for human administration.

**PET imaging**

PET imaging was performed with a Biograph mCT (Siemens, Erlangen, Germany). A list mode emission scan of 90 min duration was started simultaneously with the intravenous injection of 178 ± 6 MBq (range 165–195 MBq) \(^{18}\text{F}-\text{GE}-180\) over 10 s. Mean specific dose was 2.11 ± 0.35 MBq/kg bodyweight (range 1.62–3.11 MBq/kg). Specific dose did not differ between TSPO Ala147Thr genotypes (2.17 ± 0.39 MBq/kg, 2.00 ± 0.35 MBq/kg, and 2.10 ± 0.15 MBq/kg in HAB, MAB, and LAB, respectively, univariate analysis of variance \( p = 0.586 \)).

PET emission data were reconstructed by filtered backprojection into a sequence of 26 image frames according to the following protocol: 8 × 15 s, 3 × 60 s, 5 × 120 s, 5 × 300 s, 5 × 600 s. Voxel size was 1.57 × 1.57 × 2.00 mm\(^3\) (matrix size 200 × 200, zoom factor 2.6). A low-dose CT (100 kV, 28 eff. mAs) acquired immediately prior to the PET emission scan was used for attenuation correction. Correction for random coincidences, scatter, and radioactive decay was applied as implemented in the system software. Reconstructed images were post-filtered with an isotropic Gaussian kernel with 5 mm full-width-at-half-maximum (FWHM) [20, 21].

**Whole-blood time activity curve and input function**

The time course of radioactivity concentration in arterial whole blood during the first 15 min after start of tracer injection was measured with an automatic blood sampling device (Veenstra PBS-101, Veenstra Instruments, The Netherlands [41]). During the first 4 min, blood was drawn from the radial artery at a rate of 5 ml/min and activity concentration was

| Table 1 | Number of subjects (mean age ± standard deviation, age range) according to group and TSPO genotype |
|---------|-------------------------------------------------|
|         | LAB | MAB | HAB                              |
| Healthy subjects | 1 (63) | 1 (50) | 3 (63 ± 11, 50–71) |
| Patients after liver transplantation | 2 (64 ± 10, 57–71) | 7 (55 ± 9, 37–68) | 11 (61 ± 9, 44–77) |
measured 2 times per second. During the subsequent 11 min, blood was drawn at a rate of 2.5 ml/min and measured once every 3 s. In addition, 12 arterial blood samples were drawn manually at the midtime of the PET imaging frames 11, 14, and 17–26, that is, at 4.5, 10, 17.5, 22.5, 27.5, 32.5, 37.5, 45, 55, 65, 75, and 85 min after the start of tracer injection. Whole-blood activity concentration in the manual blood samples was measured with a well-counter (Wizard 2470, Perkin Elmer Inc., Waltham, MA, USA) cross-calibrated to the PET scanner. The automatic blood sampler was calibrated separately for each subject by comparing the activity concentration of the manual blood samples at 4.5 and 10 min with the blood sampler measurements at these time points.

The whole-blood time activity curve measured with the automatic blood sampler was corrected for delay and dispersion relative to the whole-blood time activity curve in the brain as described in the online supplementary (subsection “Correction for delay and dispersion”).

The arterial blood samples manually drawn at 4.5, 10, 17.5, 32.5, 65, and 85 min were used to determine the plasma-to-whole-blood radioactivity concentration ratio and the (parent) fraction of unmetabolized 18F-GE-180 in arterial plasma at these time points. One milliliter of arterial blood was separated into plasma and blood cell fraction by centrifugation (3’000 rcf) at 4 °C for 15 min. Two hundred fifty microliters of the plasma fraction was mixed with 250 μl ice cold methanol for degradation of proteins. Serum and proteins were separated by centrifugation (10’000 rcf) at 4 °C for 5 min. The serum fraction was transferred and cleared by a second centrifugation (10’000 rcf) at 4 °C for 10 min. The upper layer of the serum was transferred into a sample vial. The latter was measured by HPLC (Merck-Hitachi LaChrom-HPLC-system equipped with a Raytest GABI radiodetector) using a Phenomenex Chromolith RP-18e, 100 × 4.6 mm, column, and an isocratic eluent of methanol/water (60:40) at 1 ml flow. An injection volume of 200 μl was used, and fractions of 90 s (1.5 ml) were collected over 0–12 min and measured separately in the well-counter. Metabolites occurred at 2–7 min, parent compound at 8–10 min. Individual plasma-to-whole-blood time curves were fitted by a constant, because the data did not suggest another, more complex model, in line with previous findings [20, 21]. Individual parent fraction time curves were fitted by a single exponential plus constant model as described by Feeney and co-workers [21]. More precisely, individual parent fraction time curves were fitted by 1 - a * [1 - exp (−μ*t)], where a and μ are the free parameters to be optimized and t is the sampling time. Fan and colleagues used a 2-exponential linear model to describe the time course of the parent fraction of 18F-GE-180 in plasma [20]. In our data, this slightly more complex model did not improve the quality of the fit compared to the single exponential plus constant model.

The individual input function for tracer kinetic modeling was obtained as follows: input function = plasma-to-whole-blood ratio * parent fraction * whole-blood time activity curve.

**Magnetic resonance imaging**

High-resolution T1-weighted magnetization prepared rapid acquisition gradient echo (MP-RAGE) magnetic resonance images (MRI) were obtained using a 3-T Verio MRI system (Siemens, Erlangen, Germany; voxel size 1.0 × 1.0 × 1.0 mm³, echo time 2.93 ms, repetition time 1900 ms, inversion time 900 ms, flip angle 9.0°).

**Image pre-processing and tissue time activity curves**

Frame-wise correction of head motion during the PET emission scan was performed using the Realign-tool of the statistical parametric mapping software (version SPM12, https://www.fil.ion.ucl.ac.uk/spm/). Frames 7 to 26 (90 s–90 min) were included in the realignment as they provided sufficient anatomical information for reliable estimation of the rigid body transformation for realignment. The last frame was used as reference. The realignment transformation of frame 7 was also applied to frames 1–6.

The rigid body transformation to map the dynamic PET image sequence to the subject’s MRI was estimated using the Coregister-tool of SPM12 with the individual MRI as target image. The static PET image obtained by integrating the motion-corrected frames 7 to 26 was used as source image.

The individual MRI was stereotactically normalized into the anatomical space of the Montreal Neurological Institute (MNI) using the Normalize-tool of SPM12 [42]. The patient’s PET image sequence was resliced to MNI space in a single step that combined the coregister transformation to individual MRI space and stereotactical normalization from individual MRI space to MNI space.

TACs of frontal cortex, parietal cortex, temporal cortex (without mesial temporal cortex), occipital cortex, cerebellum, insula, cingulate cortex, mesial temporal cortex, precuneus, striatum, thalamus, and the superior longitudinal fasciculus were obtained by applying binary masks of these regions predefined in MNI (s. subsection “Brain regions of interest” in the online supplementary).

**Reference quantification method**

The invasive graphical Logan method [43] implemented in a custom-made MATLAB script was used to estimate the regional total distribution volume $V_T$ (unit = mL blood/cm³ tissue) from the regional tissue TACs and the individual arterial input function. The operational equation of the invasive graphical Logan method is [43].
\[
\int_0^\infty \text{TAC}(s) \, ds / \text{TAC}(t) = V_T \int_0^\infty C_p(s) \, ds / \text{TAC}(t) \\
\quad + \text{const, } t \geq t^*, \quad (1)
\]

where TAC is the time activity curve of the tissue ROI, \(C_p\) is the input function, and \(t^*\) is the time at which the plot of \(\int_0^\infty \text{TAC}(s) \, ds / \text{TAC}(t)\) versus \(\int_0^\infty C_p(s) \, ds / \text{TAC}(t)\) reaches linearity. According to its operational equation, the invasive graphical Logan method involves the area under the input function from time \(t=0\) to times \(t \geq t^*\) only. It therefore might be less sensitive than nonlinear methods (that estimate \(V_T\) by combining individual rate constants) to deviations of the population-based input function from the actual input function at early times (<\(t^*\)) at which a population-based input function might not accurately reproduce the rapid changes of individual input functions [33, 34]. This is the rationale for using the invasive graphical Logan method with population-based input functions. Fan and co-workers reported high correlation between \(V_T\) estimates obtained by the invasive Logan plot and \(V_T\) estimates obtained by the reversible 2-tissue compartment model in both HAB subjects (Pearson’s correlation coefficient \(R\) in frontal, temporal, parietal, occipital lobe, and hippocampus \(\geq 0.96\)) and MAB subjects (\(R \geq 0.85\)) [20].

The regional tissue TACs were corrected for fractional blood volume using the individual whole-blood TAC and assuming a fixed fractional blood volume of 5% [21]. Correction of tissue TACs for fractional blood volume is particularly relevant in \(^{18}\)F-GE-180 PET, because in healthy brain tissue about 20% of the PET signal is from fractional blood volume even at late time points [21]. The start of the linear fit was fixed at frame 20 so that the linear fit included the data from 30 to 90 min after tracer injection, as proposed by Zanotti-Fregonara and co-workers [44]. The fit range appropriately covered the linear part of the invasive Logan plot in all ROIs in all subjects (according to visual inspection). Some previous studies used the maximum admissible error criterion [45] to select the time start point \(t^*\) of the linear fit in graphical tracer kinetic modeling. However, this criterion tends to cause outliers [46], in particular when the same fixed maximum error is used for all ROIs [45]. In the present study, a fixed start point \(t^*\) was used in order to avoid outliers. Conventional linear regression was used to fit a straight line to the Logan plot. No effort was made to reduce noise-associated bias [47].

The invasive graphical Logan method with measured blood curves was used as reference method in this study.

**Population-based input functions**

Population-based input function approaches involve two steps [26, 32]. The first step is to generate an input function template (IFT) representing the typical shape of the input function across subjects. The second step is to generate population-based input functions (PBIF) for individual subjects from the input function template. Both steps involve scaling. In order to avoid overly optimistic performance estimates, a leave-one-out approach was used for the generation of the input function template, that is, subject \(i\) was excluded from the generation of the input function template \(IFT\), that later was used to generate the population-based input function for subject \(i\) [31, 33, 48]. More precisely

\[
IFT_i = \frac{1}{n-1} \sum_{j \neq i}^n MIF_j / SFIF^{IFT}_j 
\]

where \(n (=25)\) is the total number of subjects, \(MIF_j\) is the measured input function of subject \(j\), and the sum on the right hand side excludes subject \(i\). Normalization of the measured input function \(MIF_j\) to the scale factor \(SFIF^{IFT}_j\) is intended to reduce inter-subject variability (of the amplitude) of the input functions. Scaling to the total area under the input function was used for the generation of the input function template [26, 28, 32], that is

\[
SFIF^{IFT}_j = \int_{0}^{90 \text{ min}} MIF_j(t) \, dt 
\]

Prior to averaging the scaled individual input functions according to the right hand side of formula (2), each input function was shifted in time (by a few seconds) to achieve a common position of the peak across all scaled input functions to be averaged. The average peak position of the unshifted input functions was used as common peak position. In addition, each scaled input function was interpolated to a common time grid (every second during the first 5 min, followed by every 5 s until 15 min post injection, followed by the midtimes of the PET frames, that is, 17.5, 22.5, 27.5, 32.5, 37.5, 45, 55, 65, 75, and 85 min post injection).

The population-based input function \(PBIF_i\) of subject \(i\) was computed as

\[
PBIF_i = SFIF^{s}_i \left( \sum_{k \neq i}^{n} SFIF^{IFT}_k / \sum_{k \neq i}^{n} SFIF^{s}_k \right)^{IFT} 
\]

where \(SFIF^{s}_i\) is a simplified individual scale factor for subject \(i\). The following simplified scaling methods were tested for computation of the population-based input function according to formula (4):

- **population-based method 1**
  \[
  \text{(PB1)} : SFIF^{(1)}_i = MIF_i(t = T_0) \quad (5a)
  \]
  \[
  \text{PB2 : SFIF}^{(2)}_i = \text{mean plasma to whole blood ratio}(T_0) \text{mean fraction}(T_0) \text{WB}_i(T_0) \quad (5b)
  \]
PB3 (SUV-like) : SFIF\(^{(3)}\)
\[= \text{activity (MBq) per kg body weight administered to subject } i\]  
(5c)

Here, \(WB_i(T_0)\) is the measured whole-blood activity concentration at time \(T_0\) in subject \(i\). The time \(T_0\) of the single blood sample for scaling was selected according to the highest correlation between the activity concentration of unmetabolized \(^{18}\text{F}-\text{GE-180}\) in arterial plasma and the total area under the measured input function, that is, highest correlation of \(SFIF_i^{(t)}\) with the optimal scaling factor \(SFIF_i^{(OPT)}\) across all subjects.

In order to account for fractional blood volume also with each of the three population-based methods, a whole-blood TAC template was obtained analogous to formula (2) and scaling to the area under the individual whole-blood TAC. The scale factors for generation of the population-based whole-blood TACs analogous to formulas (5a) were

PB1 : \(SFWB_i^{(1)} = WB_i(t = T_0)\)  
PB2 : \(SFWB_i^{(2)} = WB_i(t = T_0)\)  
PB3 : \(SFWB_i^{(3)} = \text{activity (MBq) per kg body weight administered to subject } i\)  
(6c)

Each of the 3 different population-based methods was used with the invasive Logan plot to estimate \(V_T\) for each subject and each ROI. In addition, the ROI-to-whole-blood ratio (= ROI activity concentration in the last frame/whole-blood activity concentration in the 85 min blood sample) and the ROI-to-tissue-reference ratio in a sum image of the last 3 frames (60–90 min) was computed for each subject and each ROI. The cerebellum was used as pseudo-reference region, because it is amongst the brain regions with least altered \(^{18}\text{F}-\text{GE-180}\) uptake in multiple sclerosis [24]. Furthermore, it has been demonstrated that the ROI-to-cerebellum ratio of the TSPO ligand \([^{11}\text{C}]\text{PBR28}\) can serve as surrogate of \(V_T\) to detect increased TSPO availability in Alzheimer’s disease [49]. Table 2 gives a condensed overview of data required for the different simplified procedures.

**Results**

Delay and dispersion time constant of the measured whole-blood TAC relative to the image-derived whole-blood TAC in the brain were \(12.8 \pm 3.7\) s and \(3.5 \pm 1.8\) s respectively (mean over all 25 subjects). Mean plasma-to-whole-blood ratio was \(1.59 \pm 0.10\). The parent fraction of \(^{18}\text{F}-\text{GE-180}\) in arterial plasma was \(0.94 \pm 0.02, 0.91 \pm 0.04, 0.89 \pm 0.06, 0.85 \pm 0.06, 0.84 \pm 0.05, \) and \(0.80 \pm 0.08\) at \(4.5, 10, 17.5, 32.5, 65\), and \(85\) min after intravenous injection. The parameters \(a\) and \(\mu\) of the exponential plus constant model used to fit the time course of the parent fraction were \(0.23 \pm 0.17\) and \(0.072 \pm 0.061\) min\(^{-1}\), respectively. None of the parameters listed so far differed between the 14 HAB subjects and the 8 MAB subjects except the parent fraction at 10 min, which was slightly lower in HAB subjects compared to MAB subjects (0.90 \pm 0.03 versus 0.93 \pm 0.04, t-test \(p = 0.031\)). The effect did not survive correction for multiple testing.

Figure 1 shows arterial input functions and the arterial whole-blood TACs of all subjects as measured and after scaling to the total area under the individual curve. The time course of the coefficient of variance shows the reduction of inter-subject variability of input functions and whole-blood TACs by the scaling quantitatively (Fig. 1).

The blood sample at \(T_0 = 27.5\) min showed the highest correlation between the activity concentration of unmetabolized \(^{18}\text{F}-\text{GE-180}\) in arterial plasma and the total area under the measured input function (Fig. 2). Thus, the blood sample at \(T_0 = 27.5\) min was used for the population-based methods PB1 and PB2.

Mean and standard deviation of the absolute difference between the population-based and the measured input function over all 25 subjects are shown in Fig. 3. Both mean and standard deviation of the absolute difference show a peak during the first minute. This is explained by the fact that the population-based input function does not well describe the early phase of the true individual input function due to remaining inter-subject variability of the position, the height, and the width of the input function peak despite the use of a standardized injection protocol. This effect can be reduced by shifting the population-based input function such that its peak matches the peak of a whole-blood curve in the brain derived from the PET data [46]. However, the impact on the time integral of the input function at late time points (included in the linear fit of the invasive Logan plot) is negligible. As a consequence, the impact on \(V_T\) estimates is negligible, too. Individual time shift of population-based input functions was therefore not performed in this study. Mean and standard deviation of the absolute difference between the population-based input function and the measured input function reached a plateau at about 20 min post injection (Fig. 3). The plateau was lowest for the simplified scaling method PB1 and highest for the simplified scaling method PB3. The plateau for simplified scaling

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The primary finding of this study was the very strong correlation of the Logan $V_T$ estimated with population-based blood curves scaled by a single blood sample (PB1 and PB2) as well as the ROI-to-whole-blood ratio at a late time point with the reference $V_T$ across all brain regions (Table 2).

**Discussion**

The main requirement for population-based input function approaches to be suitable is that individual input functions show similar shape. In this case, individual input functions mainly differ in amplitude which can be easily accounted for by an individual scale factor. Input functions and whole-blood TACs of $^{18}$F-GE-180 fulfill this requirement to good approximation (Fig. 1), although there was an effect of the TSPO genotype: on average, there was an underestimation of the area under the input function in HAB subjects and an overestimation in MAB subjects (about 3%) by the Logan V T estimated with individual input function and whole-blood TAC (mean over all ROIs), and size of the TSPO polymorphism effect (HAB versus MAB) on the different quantitative parameters.

The main requirement for population-based input function scaling methods. However, there was no significant effect of the TSPO genotype with PB1: on average, there was an underestimation of the area under the input function in HAB subjects and an overestimation in MAB subjects (about 3%) by the Logan V T estimated with the population-based method PB1, (ii) 0.973 ± 0.007 (0.955–0.980; all $p < 10^{-10}$) for V T estimated with the population-based method PB2, (iii) 0.653 ± 0.074 (0.570–0.782; all $p < 0.003$) for V T estimated with the population-based method PB3, (iv) 0.970 ± 0.005 (0.960–0.978; all $p < 10^{-10}$) for the regional to-whole-blood ratio, and (v) 0.384 ± 0.177 (0.174–0.741; in 4 of 11 ROIs $p < 0.05$) for the regional to-cerebellum ratio.

The comparison of regional V T between ROIs and TSPO genotypes is shown in Fig. 6. The regional V T was significantly larger in HAB subjects than in MAB subjects when it was estimated by the graphical invasive Logan plot with measured blood curves or with population-based blood curves according to PB1 or PB2, or by the ROI-to-whole-blood ratio (univariate analysis of variance with TSPO polymorphism, HAB or MAB, and ROI as fixed factors: all $p < 0.0005$).

The mean ratio of the area under the population-based input function relative to the area under the measured input function is shown in Fig. 4. The mean ratio over all subjects was close to 1 for all three scaling methods, but inter-subject variability was larger for PB3 compared to PB1 and PB2. Inter-subject variability of the ratio was smallest for PB1, providing further support for PB1 as the best amongst the tested simplified scaling methods. In addition, the table gives the Pearson correlation coefficient of the simplified quantitative parameter with the reference $V_T$ across all brain regions (Table 2, requirements for quantification of $^{18}$F-GE-180 PET using the simplified methods. In addition, the table gives the Pearson correlation coefficient of the simplified quantitative parameter with the reference $V_T$.

| Quantification method | Dynamic PET imaging (≥ 90 min) | Single late blood value | HPLC analysis | Correlation of the outcome measure with reference $V_T$ | Partial effect size $\eta^2$ of the genotype effect |
|-----------------------|-------------------------------|-------------------------|--------------|---------------------------------------------|-----------------------------------------------|
| Population-based Logan plot 1 (PB1) | Required | Required | Required | 0.989 ± 0.006 | 0.200 |
| Population-based Logan plot 2 (PB2) | Required | Required | Not required | 0.973 ± 0.007 | 0.179 |
| Population-based Logan plot 3 (PB3) | Required | Not required | Not required | 0.653 ± 0.074 | 0.010 |
| ROI-to-whole-blood ratio | Not required | Required | Not required | 0.970 ± 0.005 | 0.220 |
| ROI-to-cerebellum ratio | Not required | Not required | Not required | 0.384 ± 0.177 | 0.001 |

The comparison of regional V T between ROIs and TSPO genotypes is shown in Fig. 6. The regional V T was significantly larger in HAB subjects than in MAB subjects when it was estimated by the graphical invasive Logan plot with measured blood curves or with population-based blood curves according to PB1 or PB2, or by the ROI-to-whole-blood ratio (univariate analysis of variance with TSPO polymorphism, HAB or MAB, and ROI as fixed factors: all $p < 0.0005$). There was no genotype * ROI interaction ($p = 1.000$). The mean ratio of reference $V_T$ in HAB subjects relative to reference $V_T$ in MAB subjects across all ROIs was 1.63 ± 0.14 (range 1.43–1.93). The $V_T$ based on population-based method PB3 did not show a TSPO polymorphism effect ($p = 0.130$) nor did the ROI-to-cerebellum ratio ($p = 0.556$). The partial size $\eta^2$ of the TSPO polymorphism effect (HAB versus MAB) on the different $V_T$ estimates is given in Table 2.

**Discussion**

The main requirement for population-based input function approaches to be suitable is that individual input functions show similar shape. In this case, individual input functions mainly differ in amplitude which can be easily accounted for by an individual scale factor. Input functions and whole-blood TACs of $^{18}$F-GE-180 fulfill this requirement to good approximation (Fig. 1), although there was an effect of the TSPO genotype: on average, there was an underestimation of the area under the input function in HAB subjects and an overestimation in MAB subjects (about 3%) by the Logan V T estimated with individual input function and whole-blood TAC (mean over all ROIs), and size of the TSPO polymorphism effect (HAB versus MAB) on the different quantitative parameters.

The comparison of regional V T between ROIs and TSPO genotypes is shown in Fig. 6. The regional V T was significantly larger in HAB subjects than in MAB subjects when it was estimated by the graphical invasive Logan plot with measured blood curves or with population-based blood curves according to PB1 or PB2, or by the ROI-to-whole-blood ratio (univariate analysis of variance with TSPO polymorphism, HAB or MAB, and ROI as fixed factors: all $p < 0.0005$). There was no genotype * ROI interaction ($p = 1.000$). The mean ratio of reference $V_T$ in HAB subjects relative to reference $V_T$ in MAB subjects across all ROIs was 1.63 ± 0.14 (range 1.43–1.93). The $V_T$ based on population-based method PB3 did not show a TSPO polymorphism effect ($p = 0.130$) nor did the ROI-to-cerebellum ratio ($p = 0.556$). The partial size $\eta^2$ of the TSPO polymorphism effect (HAB versus MAB) on the different $V_T$ estimates is given in Table 2.
This suggests that these parameters are useful surrogates of the Logan reference $V_T$ computed with the full arterial input function in brain PET with $^{18}$F-GE-180. Similar results have been reported by Mabrouk and co-workers who demonstrated the feasibility of TSPO quantification with $[^{18}F]$FEPPA using a input function template scaled with a single blood sample [46]. The “costs” (including burden for the patient and the staff as well as financial costs) of parameter estimation are clearly lowest for the ROI-to-whole-blood ratio at a late time point (Table 2). Thus, the ROI-to-whole-blood ratio might be a useful compromise between validity as TSPO marker and costs. This is analogous to PET with $[^{18}F]$fluorodeoxyglucose (FDG), where the (scan-time corrected) late lesion-to-blood uptake ratio shows excellent correlation with the FDG metabolic rate constant estimated by graphical analysis, better than the widely used standard uptake value (SUV) [36, 37].

The performance of population-based input functions might be improved by using two or more blood samples for individual scaling of the input function template [31, 33, 34, 46]. However, regional $V_T$ estimates obtained with the population-based method PB1 using a single blood sample at 27.5 min were strongly correlated with the regional reference $V_T$ in this study (mean Pearson coefficient over all ROIs $= 0.989 \pm 0.006$, range 0.971–0.992; all $p < 10^{-10}$). The use of additional blood samples cannot provide large improvement in this case and therefore was not tested here.

Some previous studies employed the same scaling method for the generation of the input function template from “old” subjects (with full blood sampling) as for the generation of individual population-based input functions from the input function template for “new” subjects [31, 32, 35]. In the present study, two different scaling methods were used. The input function template was generated with scaling to the total area under the input function, because this is presumably the best scaling method [26, 28, 32]. However, it requires knowledge of the full arterial input function and therefore is not suitable for generation of population-based input functions for new subjects (without full blood-sampling).

Logan $V_T$ estimated with SUV-like scaling of the population-based blood curves (PB3) and the ROI-to-cerebellum ratio showed a much weaker correlation with the...
reference VT (Table 2, Fig. 5), suggesting that these quantitative parameters are inferior as surrogate of the true Logan VT.

A secondary finding of this study was the sensitivity of the reference Logan VT to the TSPO gene polymorphism (Fig. 6). On average (across all ROIs), the reference Logan VT was 63% larger in HAB compared to MAB subjects (range 43–93%). It was smallest in LAB subjects in all ROIs (Fig. 6). The results of previous studies have been rather inconsistent with respect to allelic sensitivity of 18F-GE-180 kinetics. Fan and co-workers, performing dynamic 18F-GE-180 PET with arterial blood sampling and metabolite correction in 10 healthy volunteers, 6 HAB and 4 MAB, found regional VT to be 36–73% larger in HAB compared to MAB subjects, depending on the ROI [20]. Sridharan and co-workers, performing dynamic 18F-GE-180 PET with arterial blood sampling and metabolite correction in 6 patients with multiple sclerosis, 3 HAB and 3 MAB, found VT in whole brain (excluding lesions) to be about 70% larger in HAB compared to MAB (estimated from fig. 5e in the publication) [50]. In contrast, Feeney and co-workers,
performing dynamic $^{18}$F-GE-180 PET with arterial blood sampling and metabolite correction in 10 healthy volunteers, 5 HAB and 5 MAB, found no significant effect of the TSPO gene polymorphism on any regional $V_T$ [21]. Studies using the SUV or scaling of regional $^{18}$F-GE-180 uptake to the mean uptake in a (pseudo) reference region in a late static uptake image for semi-quantitative analysis [24, 25, 51, 52] as well as studies using a reference tissue method to model dynamic $^{18}$F-GE-180 PET data [24] in general failed to detect a TSPO gene polymorphism effect. The present study adds further evidence for allelic sensitivity of $^{18}$F-GE-180 kinetics. The fact that the simplified quantification methods based on a single blood sample (i.e., PB1, PB2, ROI-to-whole-blood ratio) were also sensitive to the TSPO gene polymorphism, whereas SUV-like scaling (PB3) and the ROI-to-cerebellum ratio were not (Fig. 6, Table 2), further supports simplified quantification based on a blood sample. It also might explain the lack of a polymorphism effect in previous studies using SUV-like scaling and/or scaling to a (pseudo) reference region (i.e., these methods are not sufficiently sensitive presumably).

The lack of a genotype * ROI interaction on the Logan $V_T$ in the present study suggests that the relation of $V_T$ between HAB and MAB subjects is more or less constant across the brain.

Estimation of the ROI-to-whole-blood ratio might be further simplified by using a late venous rather than arterial blood sample [46], because the gradient of tracer concentration between arterial and venous blood is small due to the low single-pass extraction fraction of $^{18}$F-GE-180 [20, 21] (a critical discussion of this point is given in [32]). Alternatively, arterial whole-blood activity concentration might be derived from a ROI in the descending aorta in a static PET scan acquired immediately before or after the brain scan, or both, before and after. The aorta can be delineated in the low-dose CT for attenuation correction of the aorta PET with high reproducibility across observers and software tools used for delineation [53]. If there are two scans of the aorta, before and after the brain scan, a single low-dose CT can be used for attenuation correction of both. The low-dose CT of the aorta and the low-dose CT of the brain should both be performed either before the first aorta emission scan or after the second one, in order to minimize the time delay between the brain measurement and the blood measurement. The descending aorta appears more appropriate for image-based estimation of the activity concentration in arterial whole-blood than the cavum of the left ventricle, because $^{18}$F-GE-180 shows high uptake in the myocardium causing spill-in of activity into the cavum. Due to its high $^{18}$F-GE-180 uptake, the myocardium has been suggested as potential extra-cerebral tissue reference region for $^{18}$F-GE-180 PET [54, 55].

A further secondary finding of the present study is that the total distribution volume $V_T$ of $^{18}$F-GE-180 was in general
very low throughout the whole brain (between 0.07 and 0.20 mL/cm³, Fig. 6). This is in line with previous studies in healthy human subjects [20, 21, 44, 50]. Zanotti-Fregonara and co-workers performed a head-to-head comparison of ¹⁸F-GE-180 with the TSPO PET tracer [¹¹C]PBR28 in healthy subjects and found Vₜ to be about 20 times smaller for ¹⁸F-GE-180 compared to [¹¹C]PBR28 [44]. This most likely is explained by a low permeability-surface-area-product of brain capillaries for ¹⁸F-GE-180 in line with the small rate constant K₁ for unidirectional transport of ¹⁸F-GE-180 from arterial blood to tissue even at normal cerebral blood flow (about 0.005 mL/min [21] to about 0.008 mL/min [20]). The low brain uptake of ¹⁸F-GE-180 led Zanotti-Fregonara and co-workers to question the utility of ¹⁸F-GE-180 for imaging neuroinflammation in humans (but not in rodent models). In response, Albert and co-workers summarized the evidence of the validity of ¹⁸F-GE-180 as TSPO tracer also in humans [56] (s. also [57]). Recently, Sridharan and co-workers confirmed specific binding of ¹⁸F-GE-180 in humans by a blocking study in patients with multiple sclerosis showing that in HAB subjects about 57% of Vₜ represent specific binding of ¹⁸F-GE-180 to the TSPO [50]. Simplified methods for quantitative
analysis of $^{18}$F-GE-180 as discussed here might facilitate future studies to further evaluate $^{18}$F-GE-180 in humans. A systematic comparison of 13 TSPO PET and SPECT tracers including $^{18}$F-GE-180 is given in [58].

The following limitation of this study should be mentioned. The effect of the TSPO polymorphism on the quantitative parameters was tested by comparing them between HAB and MAB subjects independent of the group (liver-transplanted patients or healthy controls). The rationale for this was that the effect of the TSPO polymorphism on $^{18}$F-GE-180 binding was expected to be larger than potential effects of the patient group. This is supported by the lack of significant differences in the distribution volume of $^{[11]}$C(R)-PK11195 between cirrhotic patients with an acute episode of clinically manifest hepatic encephalopathy and healthy subjects [59]. Age, sex, and treatment (in patient group) were also not taken into account when testing for a TSPO polymorphism effect. As a consequence, we do not recommend to use the 63% increase of $V_T$ in HAB compared to MAB observed in this study to correct for the TSPO polymorphism effect on $^{18}$F-GE-180 $V_T$ for pooling data from subjects with different genotype for combined analysis. The limitations of the polymorphism analysis do not affect the primary findings of this study from the analyses of correlation between the reference $V_T$ and the simplified quantitative parameters.

In conclusion, the present findings support the use of a population-based input function scaled with a single individual blood sample or the late ROI-to-whole-blood ratio for quantitative analysis of $^{18}$F-GE-180 PET. In the present study, an individual arterial blood sample was used for scaling. We hypothesize that the arterial blood sample can be replaced by an individual blood value derived from a late static PET scan of the descending aorta without compromising the validity of simplified quantification as a surrogate for Logan $V_T$.

Acknowledgments Open Access funding provided by Projekt DEAL. This study was supported by the German Research Foundation and by the German Federal Ministry of Education and Research (reference number: 01EO1302). It was supported partly by the German Research Foundation within the framework of FOR 2585 (project number 422182557). We like to thank the colleagues from the Department of Anaesthesiology and Intensive Care Medicine of the Hannover Medical School, especially Carolin Jung and Lukas Hinken, for their excellent assistance.

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Data availability All data generated and analyzed during this study, including the input function templates, are available for readers on request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the ethics committee of the Hannover Medical School (reference no. 6525) and the Federal Office for Radiation Protection (reference no.: ZS – 22463/2 – 2015-030). All subjects had given written informed consent for participation in the study.

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