

Cerebellum can serve as a pseudo-reference region in Alzheimer's disease to detect neuroinflammation measured with PET radioligand binding to translocator protein (TSPO)

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For submission to *Journal of Nuclear Medicine* as an Original Article, October 2014

Running Foot Line: ¹¹C-PBR28 PET non-invasive ratio method

Word count: 5030 words

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Funding: This project was funded in part by the Intramural Research Program of the National Institute of Mental Health, National Institutes of Health (IRP-NIMH-NIH), the Foundation for the NIH Biomarkers Consortium, and a fellowship award from the American Academy of Neurology Foundation to William Kreisl, under clinicaltrials.gov identifier NCT00613119 (protocol # 08-M-0066).

ABSTRACT

Alzheimer's disease (AD) is associated with increase in brain of the 18 kDa translocator protein (TSPO), which is over-expressed in activated microglia and reactive astrocytes.

Measuring the density of TSPO with PET typically requires absolute quantitation with arterial blood sampling, because a reference region devoid of TSPO does not exist in brain. We sought to determine whether a simple ratio method could substitute for absolute quantitation of binding with ^{11}C -PBR28, a second generation radioligand for TSPO.

Methods: ^{11}C -PBR28 PET imaging was performed in 21 healthy controls, 11 individuals with mild cognitive impairment (MCI), and 25 AD patients. Group differences in ^{11}C -PBR28 binding were compared using two methods. First, the “gold standard” method of calculating total distribution volume (V_T), using the two-tissue compartmental model with the arterial input function, corrected for plasma free fraction of radiotracer (f_P). Second, a ratio of brain uptake in target regions to that in cerebellum—i.e., standardized uptake value ratio (SUVR).

Results: Using absolute quantitation, we confirmed that TSPO binding (V_T/f_P): 1) was greater in AD patients than in healthy controls in expected temporo-parietal regions, and 2) was not significantly different among the three groups in cerebellum. Using the cerebellum as a pseudo-reference region, the SUVR method detected greater binding in AD patients than controls in the same regions as absolute quantification and in one additional region, suggesting SUVR may have greater sensitivity. Coefficients of variation of SUVR measurements were about two-thirds lower than those of absolute quantification, and the resulting statistical significance was much higher

for SUVR when comparing AD and healthy controls (e.g. $P < 0.0005$ for SUVR vs. $P = 0.023$ for V_T/f_P in combined middle and inferior temporal cortex).

Conclusion: To measure TSPO density in AD and control subjects, a simple ratio method SUVR can substitute for, and may even be more sensitive than, absolute quantitation. The SUVR method is expected to improve subject tolerability by allowing shorter scan time and not requiring arterial catheterization. In addition, this ratio method allows smaller sample sizes for comparable statistical significance because of the relatively low variability of the ratio values.

Keywords: Alzheimer's disease, neuroinflammation, ^{11}C -PBR28, positron emission tomography, ratio method

INTRODUCTION

Alzheimer's disease (AD) is associated with neuroinflammation characterized by activated microglia and reactive astrocytes. Because these reactive neuroimmune cells overexpress the translocator protein 18 kDa (TSPO), TSPO density has been used as a biomarker for neuroinflammation in AD and other neurological diseases (1). We recently found that TSPO binding was greater in AD patients than in age-matched controls or patients with mild cognitive impairment (MCI) who had a positive amyloid scan (2). That is, increased TSPO binding appeared to mark, and might play a pathophysiological role in, the transition from MCI to AD.

A major barrier to expanding studies that use TSPO imaging to assess the potential role of inflammation in AD is that PET measurements of TSPO density typically require 'absolute' quantitation relative to the concentration of radioligand in arterial blood. This measurement of plasma concentrations adds error to the final values, requires significant equipment and expertise, and entails arterial catheterization of the subject. A 'relative' method of measurement (e.g., one brain region compared to another) would be expected to have smaller variability than an absolute value, in part because of cancellation of global scale changes or errors in measurement of plasma concentrations. However, relative measurement requires either a reference or a pseudo-reference region. Unfortunately, a true reference region (i.e., devoid of TSPO) does not exist in brain, as TSPO is present in gray matter, white matter, vessel walls, and even choroid plexus (3, 4). A pseudo-reference region would contain TSPO but not differ between comparison groups. Such a region could, for example, be used to compare the ratio of target to pseudo-reference region in patients *vs.* controls. However, this approach requires that prior studies using absolute quantitation confirmed that the pseudo-reference region does not significantly differ between

patients and controls. To date, no study has identified or proved that a pseudo-reference region exists for second generation TSPO imaging in AD, including white matter.

Using absolute quantitation, we previously found that TSPO binding in cerebellum, measured with ^{11}C -PBR28, did not differ between AD patients and either healthy controls or MCI subjects (2). This finding is consistent with the cerebellum of AD patients being relatively spared of pathology, including inflammation (5-7), and suggests that cerebellum could be used as a pseudo-reference region as an alternative to absolute quantification.

This study sought to determine whether the method of absolute quantitation that requires arterial blood sampling could be substituted with a simple method that uses only the ratio of brain radioactivity in a target region compared to that in cerebellum. We recruited a total of 15 more subjects than in our previous report (2) and then measured TSPO binding in 25 AD patients, 21 healthy controls, and 11 patients with MCI. We compared two methods of analysis, absolute quantitation of receptor binding, which requires an arterial input function, and relative quantitation of receptor binding, which requires only PET images and was calculated as the ratio of brain uptake in target regions compared to that in cerebellum (i.e., standardized uptake value ratio (SUVR)).

MATERIALS AND METHODS

Subjects

Subjects included 21 healthy controls (15M, 6F, mean age = 55.1 ± 15.3 years), 11 MCI patients (7M, 4F, mean age = 72.2 ± 9.3 years), and 25 AD patients (11M, 14F, mean age = 63.0 ± 8.3 years). All patients were “amyloid-positive” on PET imaging with ^{11}C -Pittsburg

Compound B (PIB) based on criteria used in our earlier study (2). AD patients met updated criteria for probable AD dementia with evidence of AD pathophysiological process (8), and MCI patients met updated criteria for MCI due to high or intermediate likelihood of developing AD (9). Some subjects were included in our previously published study (2). Binding affinity status (high, mixed, or low) was determined using leukocyte binding assay as previously described (2, 10), and low affinity binders were excluded from the study. There were 7 high-affinity binders (HABs) and 14 middle affinity binders (MABs) among healthy control subjects, 5 HABs and 6 MABs among MCI patients, and 11 HABs and 14 MABs among AD patients. Both radioligand preparation and acquisition and processing of ^{11}C -PBR28 PET and magnetic resonance images are described in the Supplemental Methods. PET images were not corrected for partial volume effects (PVE).

This study was approved by the Combined Neuroscience Institutional Review Board of the NIH Intramural Research Program. All subjects or their surrogate provided written informed consent to participate.

Estimation of Binding Values

All kinetic analyses were performed with the PKIN module in Pmod 3.1 (PMOD Technologies Ltd.). The consensus nomenclature of reversible binding radioligands was followed (11).

Metabolite-corrected plasma input function and whole blood radioactivity were fitted to tri-exponential function. Time delay from the radial artery to brain was calculated with the whole blood radioactivity curve and whole gray matter TAC. Using the two-tissue compartmental model, a model curve was fitted to regional TACs with metabolite-corrected input function as

described previously (12). Finally, V_T was calculated using the rate constants (K_1 , k_2 , k_3 , and k_4) estimated from this model and corrected for plasma free fraction of radioligand (f_p).

For a non-invasive measure of ^{11}C -PBR28 binding, we created time-averaged images from 30-minute intervals of scan data (10 - 40, 20 - 50, 30 - 60, 40 - 70, 50 - 80, and 60 - 90 minutes) and converted the measured activity to SUV by multiplying subject body weight and dividing by injected activity. Regional SUV values were measured by overlaying the volume-of-interest (VOI) mask using the same regions-of-interest studied in our prior report (2), and regional SUVR values were calculated by dividing SUV value of each region by cerebellar SUV value. We sought to determine which time points best provided SUVR values, similar to the determination made for PIB by Price and colleagues (13). Unlike ^{11}C -PIB, which shows stable ratio of radioactivity in cerebellum to plasma in late time points of the PET scan (13), for ^{11}C -PBR28 this ratio increased until the end of the scan without plateauing. Therefore, to acquire an optimal time interval for SUVR, we performed a linear regression analysis between V_T/f_p values calculated from the entire 90 minutes scan and SUVR values calculated from sequential 30-minute time intervals of the image data from the combined middle and inferior temporal cortex (Supplemental Fig 1). Because the best correlation was achieved with data from 60 to 90 minutes ($r = 0.35$, $P = 0.008$), we used 60 to 90 minutes as the time interval for obtaining SUVR values for the remainder of the analysis. We also compared group binding using distribution volume ratio (DVR) by dividing the V_T of each target region by V_T of the cerebellar pseudo-reference region.

Statistical Analysis

SPSS 11.5 (SPSS Inc.) was used for the statistical analysis. Statistical correction for *TSPO* genotype was performed according to previously published methods (2). For V_T/f_P , SUVR, and DVR, *TSPO* genotype was used as a fixed factor to correct for affinity differences caused by the rs6971 single nucleotide polymorphism (SNP). For SUVR, statistical analysis was also performed without *TSPO* genotype correction. Benjamini-Hochberg's false discovery rate (FDR) with threshold P -value = 0.05 was used to correct for region-wise multiple comparisons (14). Bonferroni correction was used for multiple comparisons of each between-group comparison. Variability of binding values was defined as coefficient of variation (%COV) = standard deviation / mean x 100%. For correlative analyses, correlations were first run between the individual outcome measures and *TSPO* genotype. Standardized residuals were then plotted against each other. To determine if both V_T/f_P and SUVR values correlate with clinical severity of AD, we performed correlative analysis with Clinical Dementia Rating scale (CDR) – sum-of-boxes scores as independent variable and ^{11}C -PBR28 binding values in combined middle and inferior temporal cortex as dependent variables.

RESULTS

We used cerebellar gray matter segmented by FreeSurfer to measure uptake in the pseudo-reference tissue. The mean volume of the cerebellar gray matter of healthy controls was slightly greater than that of MCI and AD patients (HC: $96.5 \pm 12.0 \text{ cm}^3$, MCI: $95.8 \pm 9.4 \text{ cm}^3$ and AD: $91.3 \pm 9.8 \text{ cm}^3$). However, no statistically significant difference was observed in cerebellar gray matter volume between the three groups, either with or without correction for age.

No differences in V_T values were observed between the groups (Supplemental Table 1). In contrast, and consistent with results from our earlier study (2), AD patients showed greater V_T/f_P values than controls in the inferior parietal, combined middle and inferior temporal, and entorhinal cortices ($P < 0.05$; Table 1, Fig 1, and Supplemental Table 1). AD patients showed greater V_T/f_P values than MCI patients in the entorhinal and combined middle and inferior temporal cortices. All these regions survived correction for multiple comparisons. Cerebellar V_T/f_P and SUV values did not differ between controls, MCI patients, and AD patients ($P > 0.05$; Table 1 and Fig 2).

SUVR values for ^{11}C -PBR28 were greater in AD patients than controls in the inferior parietal, combined middle and inferior temporal cortices, precuneus, entorhinal, and parahippocampal cortices (Table 1 and Fig 1). With the exception of the precuneus, these regions survived correction for multiple comparisons. The SUVR values of the combined middle and inferior temporal cortex were greater in AD patients than MCI patients and survived correction for multiple comparisons. Both V_T/f_P and SUVR values in combined middle and inferior temporal cortex were positively correlated with CDR sum-of-boxes scores ($P < 0.01$; Fig 3).

The variability of SUVR values (%COV) was much lower than that of V_T/f_P values in every region and every diagnostic group (Table 2). Also, within the same *TSPO* genotype groups, the variability of SUVR values was much lower than that of V_T/f_P values (%COV of SUVR: HAB 1 - 9%, MAB 4 - 13% vs. %COV of V_T/f_P : HAB 13 - 27%, MAB 16 - 36%).

DVR values were greater in patients with AD than controls in inferior parietal lobule, combined middle and inferior temporal cortex, precuneus, hippocampus, entorhinal cortex, and parahippocampal gyrus ($P < 0.01$). DVR values were greater in AD patients than MCI patients in inferior parietal lobule, middle and inferior temporal cortex, occipital cortex, entorhinal cortex,

and parahippocampal gyrus ($P < 0.04$) (Supplemental Table 1). Variability was similar for both SUVR and DVR values after genotype correction (coefficient of variation = 7 - 21% vs. 8 - 25%).

SUVR values for ^{11}C -PBR28 were greater in AD patients than controls even without genotype correction in the inferior parietal ($P = 0.002$) and combined middle and inferior temporal cortices ($P = 0.003$). However, the statistical significance was greater ($P < 0.0005$ for two regions above), and two more regions (entorhinal and parahippocampal cortices) became significant, after correcting for *TSPO* genotype. Paradoxically, mean SUVR values in HABs were consistently lower than those in MABs in all diagnostic groups and in all regions (mean SUVR values of combined middle and inferior temporal cortex (HAB vs. MAB): 0.98 vs. 1.05 in HC, 0.98 vs. 1.10 in MCI, and 1.08 vs. 1.15 in AD; Supplemental Table 2 and Supplemental Fig 2).

DISCUSSION

This study found that simple ratio method (SUVR) can substitute for, and may even be more sensitive than, absolute quantitation for ^{11}C -PBR28 PET. Therefore, this ratio method may be promising for the clinical application of ^{11}C -PBR28 PET to study AD. One may expect the ratio method to provide results independent of *TSPO* genotype because affinity status is the same in both target regions and cerebellum. However, because genotype differences affect specific binding and not nonspecific binding, this ratio method should not completely remove the effect of *TSPO* genotype on total binding values. Therefore, *TSPO* genotype correction is still recommended.

The major limitation shared by all tested second-generation TSPO radioligands is differential affinity for the target protein caused by the rs6971 (Ala147Thr) SNP in exon 4 of the *TSPO* gene (15). Individuals without the SNP demonstrate expected affinity of radioligand for TSPO (high affinity binding). In the case of ^{11}C -PBR28, the presence of one copy of this SNP reduces specific TSPO binding by > 40% (mixed affinity binding); those homozygous for the SNP show no discernible binding at all (low affinity binding) (10). Therefore, the gold standard method of quantification for second-generation TSPO radioligands—calculating the total distribution volume (V_T) using arterial input function (12)—requires correction for affinity status to avoid underestimating TSPO density in SNP carriers. Affinity status must be determined by genetic analysis or in vivo binding assay (10, 15).

However, even within the same affinity group, significant overlap in binding values exists (10, 16), suggesting that factors other than genotype contribute to this variability (10, 17, 18). Altered TSPO expression in response to external stimuli (for instance, stress) as well as normal physiological changes may partly explain this individual variability (19, 20); it should also be noted that, even in the same subject, the contribution of these factors may change between scans. These factors may explain the low test-retest reproducibility of the TSPO radioligands ^{11}C -(R)-PK11195 and ^{11}C -DPA713 using traditional analytic methods (20, 21). In addition, the two-tissue compartmental model requires arterial catheterization to measure the concentration of parent radioligand in plasma. In addition to being invasive, arterial sampling adds a potential source of error that may increase PET data variability. The plasma free fraction may also contribute to variability in clinical PET studies. Therefore, a target-to-reference ratio approach may be preferable to absolute quantification with compartmental modeling, both for eliminating individual variation of physiologic TSPO expression and avoiding errors in

measuring plasma concentration of radioligand. Our ratio approach appears to at least partly reduce the variability caused by differences in *TSPO* genotype as well. In the present study, the %COV of V_T/f_P was higher than 30% in all regions. This high variability requires relatively large sample sizes to detect statistically significant differences between groups. However, the variability of the SUVR was much lower (less than 22%), and we expect this ratio method to increase statistical power to detect differences in *TSPO* binding in clinical studies of AD.

Errors in measuring the free fraction of radioligand may also contribute to high variability in clinical PET studies. Because only free radioligand enters the brain, correcting V_T for f_P should theoretically increase the accuracy of PET measurement of receptor density. However, correcting for f_P may introduce noise, thereby reducing precision. In our study, we found no difference in %COV between V_T and V_T/f_P values after genotype correction (36 - 74% vs. 34 - 71%). Using V_T/f_P thus did not reduce precision, suggesting that the theoretically more accurate V_T/f_P should be used for absolute measurement of ^{11}C -PBR28 binding.

For a ratio method to be clinically useful in PET studies, the reference region should be relatively unaffected by disease pathology in terms of the density of the target protein. Although diffuse amyloid plaques with surrounding microglia can be found in the cerebellum in advanced AD, the morphology of the cerebellar microglia differs from that of typical activated microglia in neocortex, and the cerebellum is relatively spared from neurodegeneration (5-7). Therefore, we can reasonably assume that pathological increases in *TSPO* are much lower in cerebellum than in cortical gray matter regions most affected by AD, and that the cerebellum can serve as the best reference region in AD (22). This argument is supported by the similar values observed for V_T/f_P and SUV in cerebellum among the healthy controls and MCI and AD patients in this and in our previous study (2).

When using ^{11}C -PBR28, both DVR and SUVR discriminate AD patients from MCI patients and controls. Although the DVR method has the advantage of using the full kinetics of the radioligand, SUVR is more practical because it does not require arterial catheterization and allows for shorter scan time.

An earlier study using the second-generation TSPO radioligand ^{11}C -DAA1106 found greater binding in AD patients than controls in several regions, including cerebellum (23). In that study, cerebellar binding potential (BP_{ND}) was 14% greater in AD patients than in controls (10 AD patients vs. 10 controls). In our larger study (25 AD patients vs. 21 controls) we found no difference in binding (using $V_{\text{T}}/f_{\text{P}}$) in cerebellum. This discrepancy could be due to several factors, including the outcome measure used and the number and characteristics of the study subjects. However, the difference in the TSPO radioligand used could also be a factor. Therefore, studies using TSPO radioligands other than ^{11}C -PBR28 should compare cerebellar binding using absolute values (e.g., $V_{\text{T}}/f_{\text{P}}$) between patient and control groups before using this SUVR method.

Our approach in this study was similar to that of Coughlin and colleagues, who showed that normalizing the V_{T} values of regions for ^{11}C -DPA713 to that of total gray matter lowered variability and improved test-retest reproducibility (20). This normalization also removed the effect of *TSPO* genotype differences. However, using global gray matter as the reference region means that binding in the target region is represented in both the numerator and denominator. Therefore, this approach may underestimate binding in target regions, particularly relatively preserved areas in AD, and may produce unexpected results of decreased binding in those regions in AD patients.

Unlike the gray matter normalization by Coughlin and colleagues, SUVR with cerebellar pseudo-reference region did not completely ameliorate the *TSPO* genotype effect as larger

differences between AD patients and controls were detected after correcting ^{11}C -PBR28 binding values for affinity status. Therefore, we recommend genotype correction when using the SUVR method. In this study, *TSPO* genotype had a paradoxical effect on the ratio method of analysis, resulting in MABs having larger SUVR values than HABs. We are not certain if this unexpected finding represents a true result or not. Additional studies with larger sample sizes are needed to clarify the significance of this finding.

One of the most notable results of this study is clear replication of our previous findings. In our earlier study (2), we found that the AD patients showed greater V_T/f_P values in the inferior parietal, entorhinal, and combined middle and inferior temporal cortices than controls when using PET data not corrected for PVE. By including 15 additional subjects (42 subjects in the previous vs. 57 in the current study), we found greater ^{11}C -PBR28 binding in AD patients in the same regions. Moreover, by using the SUVR method we found that AD patients showed greater a larger number of regions, with similar distribution to that seen in our previously reported results using ^{11}C -PBR28 with two-tissue compartmental model and PVE-corrected data. Using SUVR values did not reduce the correlation between clinical severity and ^{11}C -PBR28 binding seen using V_T/f_P . Although we did not include PVE correction in this study, we would expect to see even larger differences between AD patients and controls.

Because MCI patients with amyloid-positivity on PET are at high risk for developing dementia due to AD (24), the increased neuroinflammation observed with ^{11}C -PBR28 PET may be a marker for conversion to clinical AD (2). We hope this SUVR method for ^{11}C -PBR28 PET could prove useful for testing anti-inflammatory agents in AD (25).

CONCLUSION

A simple ratio method (SUVR) can substitute for, and may even be more sensitive than, absolute quantitation for detecting regions with increased binding to TSPO in AD. This method reduces variability and has the advantages of not requiring arterial sampling and allowing for shorter scan time. *TSPO* genotype correction was still required to increase sensitivity. This method is expected to improve subject tolerability for ^{11}C -PBR28 PET studies in AD, particularly longitudinal studies, and increase the power necessary to detect group differences. The method also needs to be replicated in larger samples of AD patients before it can be widely used. For other diseases, application of this method will first require validation with gold standard methods of quantification that binding in cerebellum is not affected by the disease itself.

ACKNOWLEDGEMENTS

This project was funded in part by the Intramural Research Program of the National Institute of Mental Health, National Institutes of Health (IRP-NIMH-NIH), the Foundation for the NIH Biomarkers Consortium, and a fellowship award from the American Academy of Neurology Foundation to William Kreisl, under clinicaltrials.gov identifier NCT00613119 (protocol # 08-M-0066). Ioline Henter provided excellent editorial assistance. All authors have no conflicts of interest to disclose, financial or otherwise.

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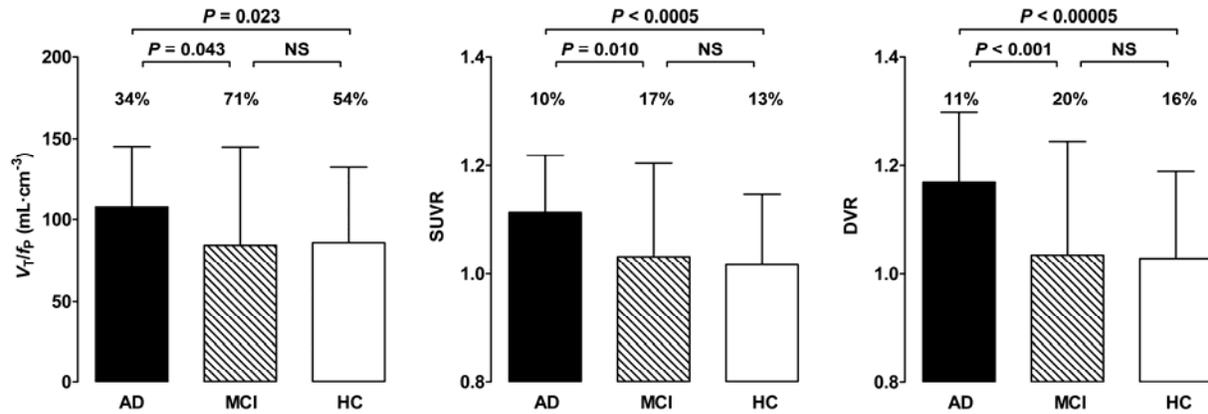


FIGURE 1. In the combined middle and inferior temporal cortex, total distribution volume corrected for free fraction of radioligand (V_T/f_P), standardized uptake value ratio (SUVR), and distribution volume ratio (DVR) values were greater for Alzheimer’s disease (AD) patients than for mild cognitive impairment (MCI) patients or controls. Error bars denote mean \pm SD. The SD bars have similar heights for V_T/f_P and SUVR due to different scales on the two y-axes. The coefficient of variation of V_T/f_P was three to four times greater than that for SUVR and DVR, as shown by coefficient of variation (%COV) values above the SD bars.

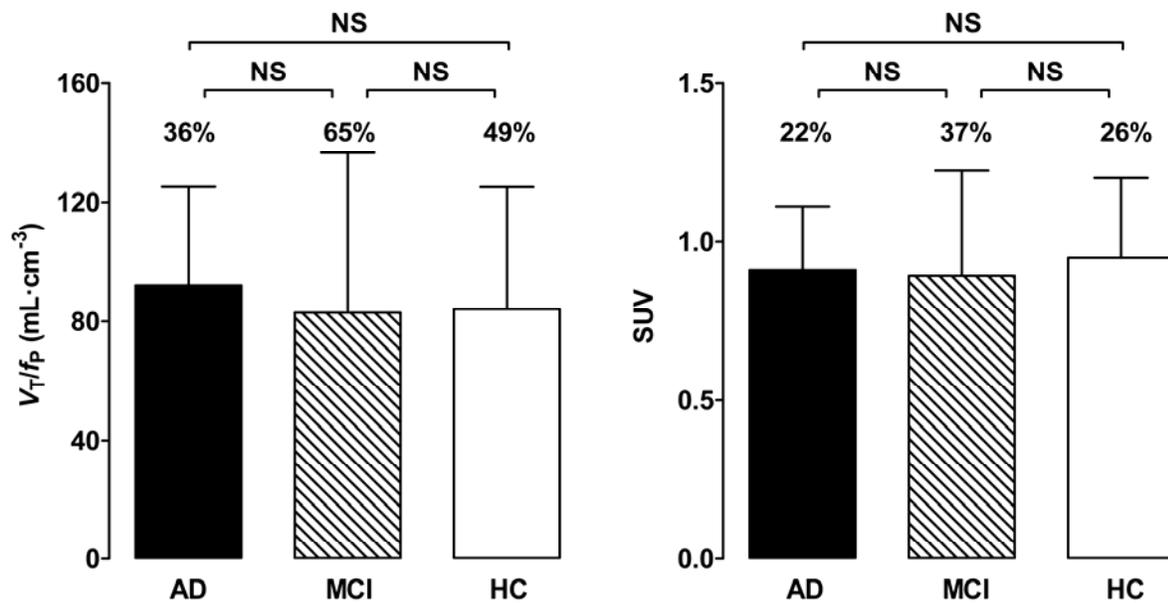


FIGURE 2. In the cerebellum, ¹¹C-PBR28 binding did not differ between controls and patients with mild cognitive impairment (MCI) or Alzheimer’s disease (AD). ¹¹C-PBR28 binding values in total distribution volume corrected for free fraction of radioligand (V_T/f_p) and standardized uptake value (SUV) are shown. Error bars denote mean ± SD. Coefficient of variation (%COV) values are shown above the vertical bars.

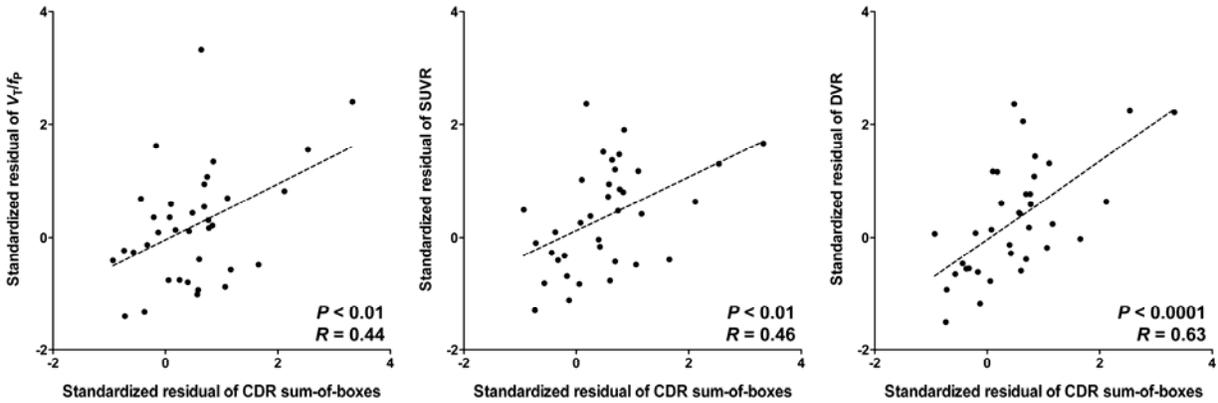


FIGURE 3. Correlation between the binding values and the severity of cognitive impairment measured by the sum-of-boxes of clinical dementia rating (CDR) score.

Total volume of distribution corrected for plasma free fraction (V_T/f_p), standardized uptake value ratio (SUVR), and distribution volume ratio (DVR) of combined middle and inferior temporal cortex similarly correlated with the severity of cognitive impairment. Non-invasive ratio method did not deteriorate correlation with clinical severity.

TABLE 1. Effect of quantification method on level of statistical significance in detecting differences in ^{11}C -PBR28 binding

	<i>P</i> -value (AD vs. HC)		
	V_T/f_P	SUVR	DVR
Inferior parietal	0.028	< 0.0005	< 0.00005
Middle & inferior temporal	0.023	< 0.0005	< 0.00005
Precuneus	NS	0.048*	0.006
Entorhinal	0.048	0.009	0.001
Parahippocampal	NS	0.006	0.009

AD = Alzheimer's disease; HC = healthy control; V_T/f_P = total distribution volume/free fraction of radioligand; SUVR = standardized uptake value ratio; DVR = distribution volume ratio; NS = not significant. *P*-values were derived from univariate ANOVA using diagnosis and TSPO genotype as fixed factors.

* Region did not survive region-wise correction for multiple comparisons.

TABLE 2. Effect of quantification method on variability of ¹¹C-PBR28 binding values

	Coefficient of variation (%COV)								
	AD			MCI			HC		
	V_T/f_P	SUVR	DVR	V_T/f_P	SUVR	DVR	V_T/f_P	SUVR	DVR
Inferior parietal	34.5	10.4	10.8	70.0	18.0	19.3	53.4	14.2	15.2
Middle & inferior temporal	34.3	9.5	11.0	71.4	16.7	20.3	53.5	12.9	15.5

AD = Alzheimer's disease; HC = healthy control; MCI = mild cognitive impairment; V_T/f_P =

total distribution volume/free fraction of radioligand; SUVR = standardized uptake value ratio;

DVR = distribution volume ratio

Data are presented as %COV (= standard deviation / mean x 100%) with ¹¹C-PBR28 binding values corrected for *TSPO* genotype.