

# 3D-SSP analysis for amyloid brain PET imaging using <sup>18</sup>F-florbetapir in patients with Alzheimer's dementia and mild cognitive impairment

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## ABSTRACT

**Introduction:** The aim of this study is to use 3D-SSP and a population-comparable normal database to investigate the associations between amyloid deposition detected by <sup>18</sup>F-florbetapir PET and neurocognitive performance of participants with mild cognitive impairment (MCI) and Alzheimer's disease (AD).

**Materials and Methods:** <sup>18</sup>F-florbetapir PET and <sup>18</sup>F-FDG PET imaging was prospectively performed on 78 subjects (20 cognitively healthy controls [HC], 27 MCI patients, and 31 AD patients) within 6 weeks of their neurocognitive assessments. The PET datasets from 19 HCs were used to create an NBD. The 3D-SSP analysis and Z-score mapping of <sup>18</sup>F-florbetapir accumulations in the brain were further staged based on their accumulation patterns. Global and regional standard uptake value ratios (SUVRs) of <sup>18</sup>F-florbetapir were calculated using the cerebellar cortex as the normalised region. The relationships between the <sup>18</sup>F-florbetapir PET results, the clinical diagnoses and Thai Mini-Mental State Examination (TMSE) scores were determined.

**Results:** There was high agreement between the visual assessment results and the semiquantitative analysis ( $\kappa = 0.793$  and  $0.845$ ). The stages of amyloid deposition were consistent with neurocognitive status across participants. Significantly higher SUVRs were found in AD than MCI and HC. Visual assessment and stage were not significantly correlated with TMSE scores. A significant negative correlation between the SUVRs and TMSE scores was partially demonstrated in MCI and AD, but not HC.

**Conclusions:** 3D-SSP analysis of <sup>18</sup>F-florbetapir PET provides special patterns and intensity of beta amyloid accumulation semi-quantitatively that are associated with the diagnosis and neurocognitive performances in MCI and AD patients.

## KEYWORDS:

<sup>18</sup>F-florbetapir; amyloid; brain PET; Alzheimer's disease; NEUROSTAT; 3D-SSP

## INTRODUCTION

Alzheimer's disease (AD) creates a significantly negative impact on the quality of life of patients and their families and is a major socioeconomic burden. Conventional imaging

modalities have a relatively limited ability to accurately differentiate the types of dementia. Glucose hypometabolism detected from <sup>18</sup>F-fluorodeoxyglucose (FDG) positron emission tomography (PET) study provides characteristic patterns in AD, frontotemporal lobe dementia (FTD) and dementia with Lewy Bodies (DLB). However, there are overlapping of abnormalities. The accumulation of amyloid beta (A $\beta$ ) plaques in the brain is one of the important factors associated with the loss of synapses and neuronal degeneration during the preclinical phase of AD,<sup>1</sup> and it is required for a pathological diagnosis of AD.<sup>2</sup> There has been a growing acceptance of the use of PET imaging of amyloid beta deposition in the brain for several purposes, namely, to enable the early identification of subjects who might be at risk of developing AD dementia, to select patients for amyloid-clearing therapies and to evaluate therapeutic efficacy.<sup>3-6</sup> Visual interpretation of A $\beta$  PET images using binary "positive" and "negative" scales is routinely performed. However, equivocal cases may present,<sup>6</sup> and the correlation between binary-scale interpretation and neurocognitive status is as yet conclusive.<sup>5,7-9</sup> Semiquantitative analysis of A $\beta$  PET images using standard uptake value ratios (SUVRs) shows the advantage of providing consistency in interpretation, diagnostic and prognostic classification, and objective evaluation of longitudinal changes.<sup>10-12</sup> The recent development and integration of toolboxes in free software has enabled the automation of the calculation of SUVRs of brain PET images.<sup>13-14</sup> Nevertheless, the use of such tools is still mainly limited to research settings. The need for individual structural magnetic resonance imaging (MRI) images also does not allow some techniques to be used for patients who cannot undergo MRI.

A semiquantitative image analysis using 3-dimensional stereotactic surface projections (3D-SSP) in PET neuroimaging studies has been shown to improve the accuracy of diagnoses of different neurological abnormalities.<sup>15</sup> Using 3D-SSP Z-score maps, the patterns of abnormal radiotracer distribution in a subject's brain relative to a normal subject database may facilitate diagnosis. However, the results from 3D-SSP analysis of amyloid beta PET studies are relatively limited<sup>16,17</sup> and can differ with the normal controls and radiopharmaceuticals used. Five stages of amyloid deposition were proposed in a recent study on the frequency of the regional distribution of <sup>18</sup>F-florbetapir PET images obtained from Alzheimer's Disease Neuroimaging Initiative (ADNI)

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data. The stages were based on regional brain involvement, and they were found to be very consistent with clinical diagnoses, CSF A $\beta$ 42 levels and some neurocognitive test results.<sup>18</sup>

In the current study, we used semiquantitative data from <sup>18</sup>F-florbetapir PET images of the brain in patients with AD and mild cognitive impairment (MCI). The reference database of <sup>18</sup>F-florbetapir PET images was acquired from a cognitively normal, elderly, Thai population (Thai NDB). We demonstrated a high association between the amyloid PET results and clinical diagnoses. Negative correlations between amyloid PET results and the TMSE scores were also partly observed.

## MATERIALS AND METHODS

This single centre study was approved by the Institutional Review Board as a part of the Amyloid PET Project (COA: Si137/2015). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

### Subjects

This study enrolled 78 subjects who had visited the Geriatric Clinic, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. The participants were aged 60 and above, and comprised 20 cognitively healthy controls (HCs), 27 patients with clinically diagnosed MCI, and 31 patients with clinically diagnosed AD. Formal consent was given by the subjects or their legally authorised representatives. Normal elderly subjects were also recruited via a poster advertisement. The <sup>18</sup>F-fluorodeoxyglucose (FDG) and <sup>18</sup>F-florbetapir PET were performed on all subjects between September 2016 and June 2018, and within 6 weeks of their neurocognitive assessments. Exclusion criteria were as follows: an unstable medical condition, seropositivity for HIV or AIDS, alcoholism, drug abuse, primary or metastatic brain cancer, significant brain lesions, a history of amyloid-targeting medication usage, or a lack of willingness to follow the study protocol. The neurocognitive tests performed on all of the subjects comprised the Thai Mini-Mental State Examination (TMSE),<sup>19</sup> Clinical Dementia Rating (CDR) Scale,<sup>20</sup> Alzheimer's Disease Assessment Scale–Cognitive subscale (ADAS-COG),<sup>21</sup> and Thai Activities of Daily Living (ADLs).<sup>22</sup> The study used established criteria from of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)<sup>23</sup> and the International Working Group on Mild Cognitive Impairment<sup>24</sup> to define participants with AD, MCI and cognitively HC based on their clinical complaints, TMSE and CDR scores as of followings;

- AD: Subjects with dementia symptoms compatible with fulfilled criteria for probable AD; TMSE < 26; CDR  $\geq$  0.5.
- MCI: Subjects with a subjective memory complaint reported by the patient, a family member or a clinician, plus impairment on objective cognitive tasks and/or evidence of decline over time on objective cognitive tasks,

with preserved activities of daily living; TMSE 24-30, CDR = 0.5.

- HC: Subjects without neurological or psychiatric illness, no MCI or dementia symptoms and normal activities of daily living; TMSE 24-30; CDR = 0.

### PET image acquisition

The in-house productions of the <sup>18</sup>F-florbetapir tracer and imaging protocols were as previously described.<sup>25,26</sup> All subjects underwent both <sup>18</sup>F-FDG and <sup>18</sup>F-florbetapir brain PET/CT scans with at least a 24-hour interval between the scans, acquired in 3D-mode on a Discovery STE PET/CT scanner (GE Healthcare, Milwaukee, Wis., USA). The acquisition and reconstruction techniques followed the scanner-specific protocols from ADNI 2 and were stated in our previous work.<sup>26,27</sup> The <sup>18</sup>F-FDG PET/CT scans were acquired over approximately 30 minutes following a 4.5–5.5 mCi <sup>18</sup>F-FDG injection and scanned for 30 minutes. The amyloid PET/CT scans were acquired over approximately 50 minutes following 8–10 mCi <sup>18</sup>F-florbetapir injection and scanned for 20 minutes. The subjects were positioned in the scanner using a laser light beam to ensure proper head alignment, and a computed tomography scan was acquired prior to the PET imaging for attenuation correction. Immediately after the acquisition, the images were reconstructed and corrected for scatter and attenuation using commercial software packages and inspected for adequacy of count statistics and absence of head motion. Summed images from the reconstructed <sup>18</sup>F-FDG PET/CT data and the reconstructed amyloid PET/CT data were generated for each subject, after excluding any image frames in which head motion was detected.

### Image analysis

For each PET scan, the anonymised and summed DICOM image files were converted to one analysis format file using ImageJ 1.51s software (National Institutes of Health, Bethesda, Md., USA; available at <http://imagej.nih.gov/ij>). Each converted file was further processed using Neurological Statistical Image Analysis Program (NEUROSTAT/3D-SSP software, University of Utah, Salt Lake City, Utah, USA) to transform the reconstructed images to the stereotactic coordinate system and to co-register the <sup>18</sup>F-FDG and <sup>18</sup>F-florbetapir PET/CT images using previously validated methods, described as follows.<sup>15-17</sup> Each <sup>18</sup>F-FDG and <sup>18</sup>F-florbetapir PET/CT image generated by the NEUROSTAT software underwent quality control for alignment and coregistration before interpretation. The regional activities of the <sup>18</sup>F-florbetapir were extracted from the cortical grey matter to the surface of the template using the 3D-SSP method in the same manner as for <sup>18</sup>F-FDG PET/CT. The <sup>18</sup>F-florbetapir SUVRs were calculated from the cortical activity in particular brain regions normalised by the average activity of cerebellar cortex (CBL) using NEUROSTAT scaling procedures. The average values of the bilateral hemispheric global and regional cerebellar normalised SUVRs were calculated in accordance with the most validated regions for amyloid deposition found by a previous study<sup>28</sup>, namely, the frontal cortex, parietal cortex, temporal cortex, occipital cortex, anterior cingulate, and posterior cingulate. In this study, <sup>18</sup>F-FDG PET/CT images were used mainly to assist in coregistration amyloid PET/CT images to the standard brain

template. The brain glucose metabolism in all subjects were also evaluated and classified as AD-liked, FTD-liked and other patterns (normal, undetermined or DLB)<sup>29</sup> to support or explain their amyloid PET/CT results, which were further discussed.

The spatially normalised <sup>18</sup>F-FDG and amyloid PET/CT scans from 20 cognitively normal volunteers (HCs; age 60–82 years) were used to create a NDB for each PET/CT study. The original scans of those HCs had been interpreted in consensus by visual assessment as being negative for both glucose hypometabolism and amyloid deposition, as per standard guidelines.<sup>6</sup> The PET/CT images of <sup>18</sup>F-florbetapir accumulation in the brain of each AD and MCI patient were compared with the NDB. The Z-scores ( $Z = [\text{voxel}_{\text{subject}} - \text{voxel mean}_{\text{NDB}}] / \text{voxel standard deviation}_{\text{NDB}}$ ) were calculated on a voxel basis; the cortical activities were extracted to predefined surface pixels using the 3D-SSP technique, and the Z-score maps were automatically generated by computing the intensity normalised to the cerebellar cortex.<sup>16-17</sup>

The original transaxial images and 3D-SSP Z-score maps interpretations were performed in consensus by 2 experienced nuclear medicine specialists who were trained in <sup>18</sup>F-florbetapir PET interpretation (T.T. and C.S) without knowledge of the related clinical information. In equivocal cases, the images and maps were confirmed by a senior neuroimaging expert (S.M.). The visual assessments were classified as “positive” or “negative,” following standard guidelines.<sup>6</sup> To evaluate abnormally increased cortical amyloid depositions, positive Z-score maps displayed on a colour-coded scale—which reflected positive tracer uptake deviations relative to the norm—were used for interpretation. The positive Z-score map pattern of each participant was staged as 0–IV, according to the recently reported criteria<sup>18</sup>: Stage 0, no involvement; Stage I, basal part of the temporal lobe, the anterior cingulate gyrus and the parietal operculum; Stage II, wide parts of the temporal, frontal and parietal associative cortex; Stage III, primary sensory-motor cortices and anterior medial temporal lobe; and Stage IV, posterior medial temporal lobe and the striatum. An abnormal amyloid PET/CT was considered in the regions with a Z score level of  $\geq 2$ , which corresponds to the green colour and above on the colour scale bar (Figure 1). Each stage was then classified as “negative” (Stages 0–I) or “positive” (Stages II–IV) to assess the correlation with the binary visual interpretation. Abnormal amyloid depositions in the areas of a higher stage without involvement at the areas of a lower stage were deemed “Unstageable”. Comparisons were then made of the results from each interpretation technique and the visual assessment of the original images and the semiquantitative amyloid PET results.

#### Statistical analyses

Statistical analyses were performed using PASW Statistics for Windows (version 18.0; SPSS Inc., Chicago, Ill., USA). Descriptive statistics were used to characterise the demographic and baseline characteristics of the study subgroups. Chi-squared tests and one-way ANOVA were applied for statistical comparisons between the study subgroups, followed by a post hoc test with pairwise comparisons. Agreement between the visual and

semiquantitative amyloid PET/CT results were expressed in terms of Cohen’s Kappa and percentage agreement. Pearson correlation and Spearman’s correlation analyses were used to evaluate the relationships between the PET/CT results and the TMSE scores. Statistical significance was defined as  $P < 0.05$ .

## RESULTS

The automatic co-registration of both brain PET/CT datasets was completely successful for 77 of the 78 subjects. The one unsuccessful case involved an individual with mild AD, for whom significant co-registration errors occurred. The baseline characteristics of the 77 study subjects subsequently analysed are detailed in Table I. There were no significant differences in the age, sex, or education levels of the study subgroups. Significant differences ( $p < 0.05$ ) were observed for all neurocognitive scores, with better neurocognitive performances for the HCs than the AD subjects, and for the MCI than the AD subjects. Only the CDR score was significantly lower for the MC than the HC subgroup; though the other tests showed a lower cognitive performance for the MCI subgroup, the differences did not reach statistical significance.

The results from the visual assessments were also consistent with the clinical diagnoses: negative in 19/20 of the HCs (95%), while positive in 26/30 (87%) of the AD and 13/27 of the MCI (48%) subjects. Likewise, the stages of amyloid deposition were consistent with the clinical diagnoses: 19/20 (95%) of the HCs had negative results (Stage 0), while 24/30 (80%) of the AD and 9/27 (33.3%) of the MCI subjects had positive results (Stages II–IV). An additional 3.3% of the AD and 3.7% of the MCI participants showed a Stage I pattern, with amyloid deposition only at the anterior cingulate; this was considered negative by the definitions used in this study. There was a high agreement of amyloid positivity between the visual assessments and the Z-score maps (92.2% agreement;  $\kappa = 0.845$ ; 95% CI, 0.785–0.905), and between the visual assessments and the global SUVRs (89.6% agreement;  $\kappa = 0.793$ ; 95% CI, 0.724–0.862). The main discordant amyloid PET/CT results were found in 6 patients (4 MCIs; 2 ADs), whose results were considered positive by visual assessment but negative by the Z-score map staging and SUVRs.

Significant differences were observed between the global and regional SUVRs of the HCs and AD subjects, and of the AD and MCI subjects: the AD subgroup had significantly higher SUVRs than the MCI subgroup ( $p < 0.001$ – $0.003$ ) and the HC subgroup ( $p < 0.001$ ; Figure 2). However, there were no significant differences between the regional nor the global SUVRs of the HC and MCI subgroups. From an ROC curve analysis, the best cutoff of cerebellar normalised SUVR to discriminate between HC and AD was 1.15, with a sensitivity of 83.3% and a specificity of 90%. The results of the different methods used to interpret the <sup>18</sup>F-florbetapir PET/CT images for all clinical diagnoses are illustrated in Figure 3. Negative correlations between the results of the <sup>18</sup>F-florbetapir PET/CT imaging and the TMSE scores were observed in all subgroups, regardless of the interpretation method. However, the correlations from the visual assessments and the staging by the Z-score map patterns were not statistically significant.

**Table I: Demographics and characteristics of subjects of clinical diagnostic groups**

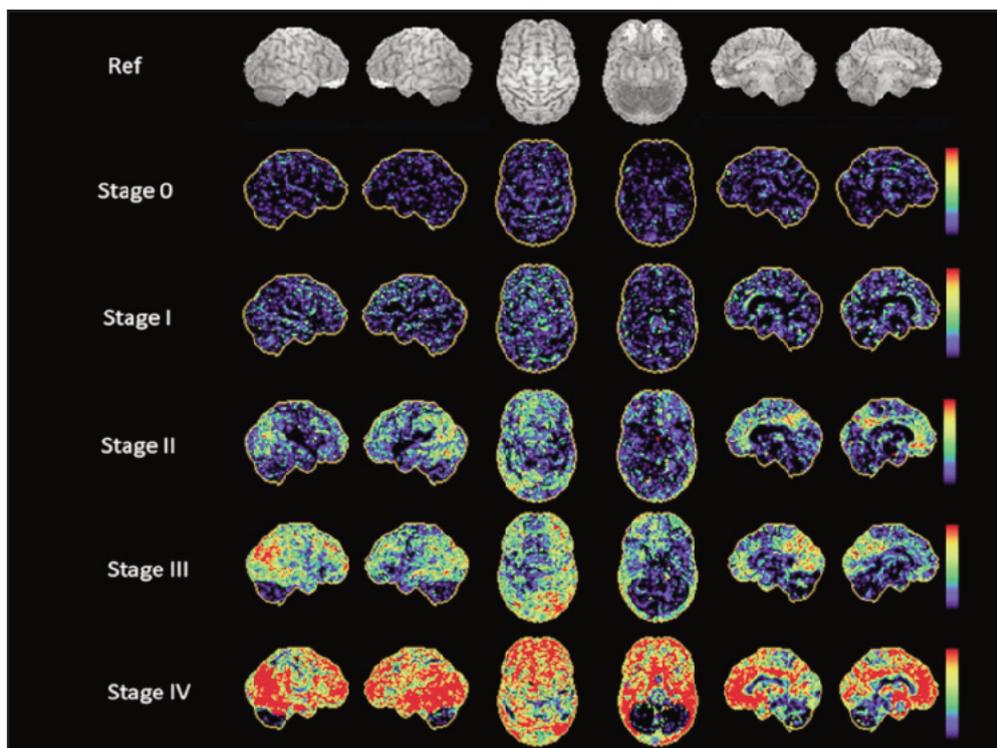
	HC (n = 20)	MCI (n = 27)	AD (n = 30)	P-value
Age, years, mean (SD)	69.35 (5.10)	68.67 (6.09)	71.70 (6.31)	0.139
Sex (% female)	65%	48.15%	50%	0.468
Education, years, mean (SD)	11.85 (5.76)	14.70 (4.50)	13.13 (10.66)	0.455
Onset, months, mean (SD)	N/A	19.96 (10.55)	38.33 (17.69)	< 0.001 <sup>a</sup>
TMSE, score, mean (SD)	27.65 (1.81)	27.15 (1.61)	22.07 (4.65)	< 0.001 <sup>a,b</sup>
CDR, score, median (IQR)	0	0.5 (0)	0.75 (0.5)	< 0.001
ADAS-COG, score, mean (SD)	5.18 (2.02)	8.91 (4.07)	19.67 (9.92)	< 0.001 <sup>a,b</sup>

Abbreviations: HC, cognitively healthy control; MCI, mild cognitive impairment; AD, Alzheimer’s disease; TMSE, Thai Mini-Mental State Examination; CDR-SB, Clinical Dementia Rating–Sum of Boxes, ADAS-COG, Alzheimer’s Disease Assessment Scale–Cognitive subscale; SD, standard deviation; IQR, interquartile range; a, significant difference found between HC and AD; b, significant difference found between AD and MCI

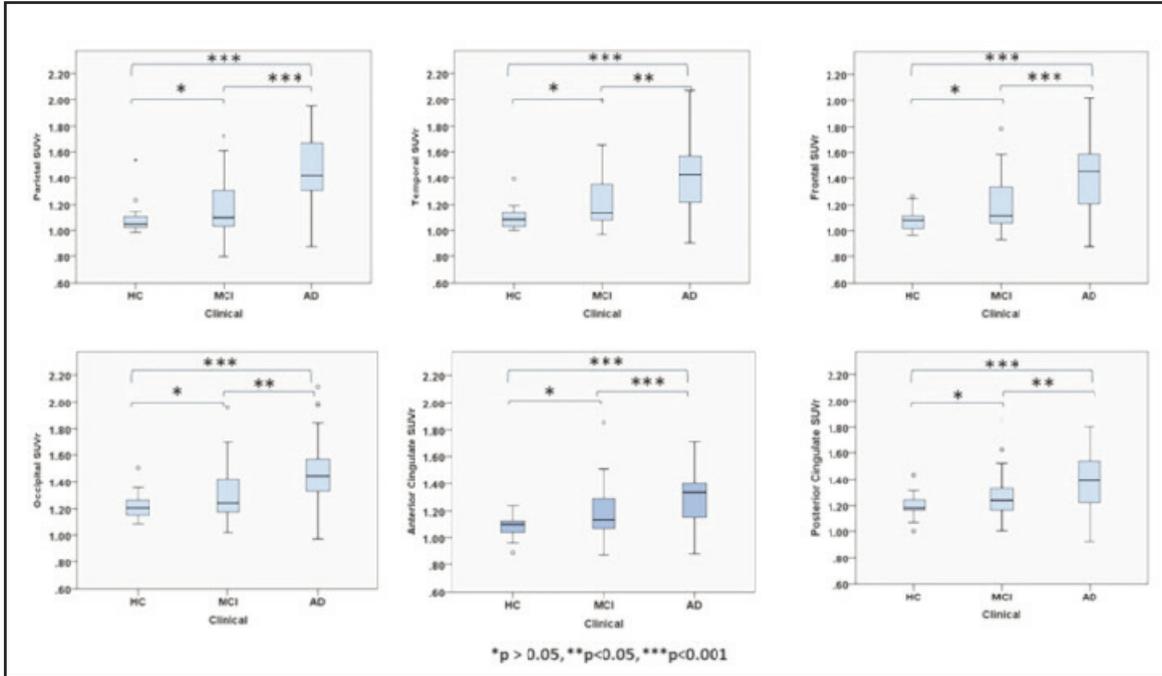
**Table II: Correlation between visual assessments, stages from Z-score map patterns, and global cortical and regional SUVRs obtained from 18F-florbetapir PET data, with neurocognitive performance (evaluated with the TMSE scores)**

Method	Region	Correlation coefficient		
		Method	Region	Correlation coefficient
Visual assessment		-0.387	-0.190	-0.154
Stage		-0.387	-0.250	-0.239
SUVR	Cerebral cortex	-0.323	-0.347*	-0.216
	Parietal	-0.328	-0.343*	-0.142
	Temporal	-0.366	-0.341*	-0.158
	Frontal	-0.286	-0.381*	-0.209
	Occipital	-0.346	-0.143	-0.350*
	Anterior cingulate	-0.071	-0.413*	-0.039
	Posterior cingulate	-0.340	-0.316	-0.070

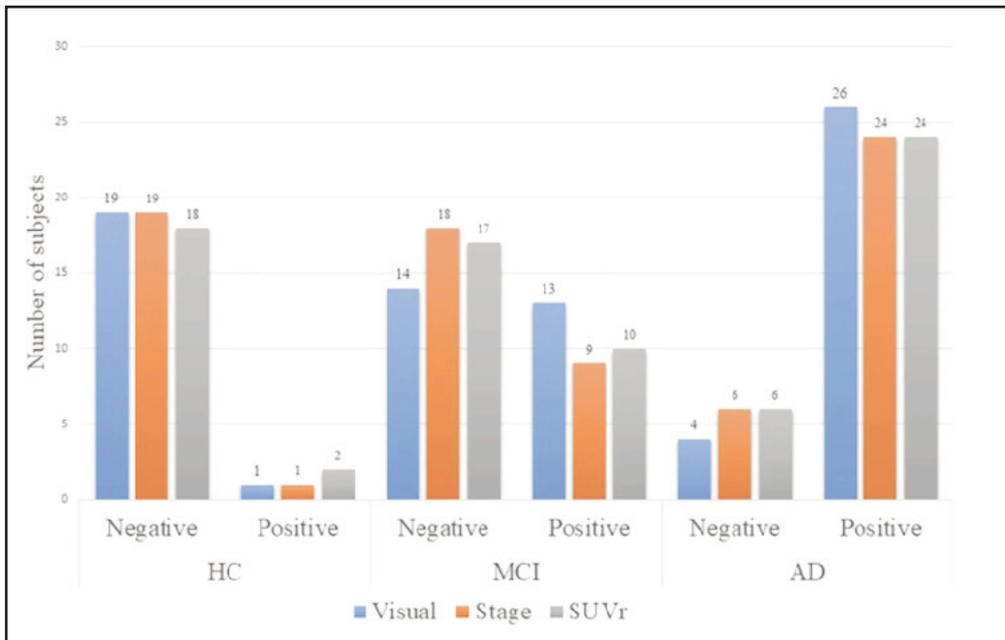
\* P-value of < 0.05



**Fig. 1:** In vivo staging of amyloid beta deposition using individual Z-score image patterns from 18F-florbetapir PET, compared with normal database applied from previously proposed staging system, according to regional amyloid deposition.15 From left to right, the image views are right lateral, left lateral, superior, inferior, right medial and left medial. The range of the Z-score colour codes was set from 0 (black) to 5 (red).



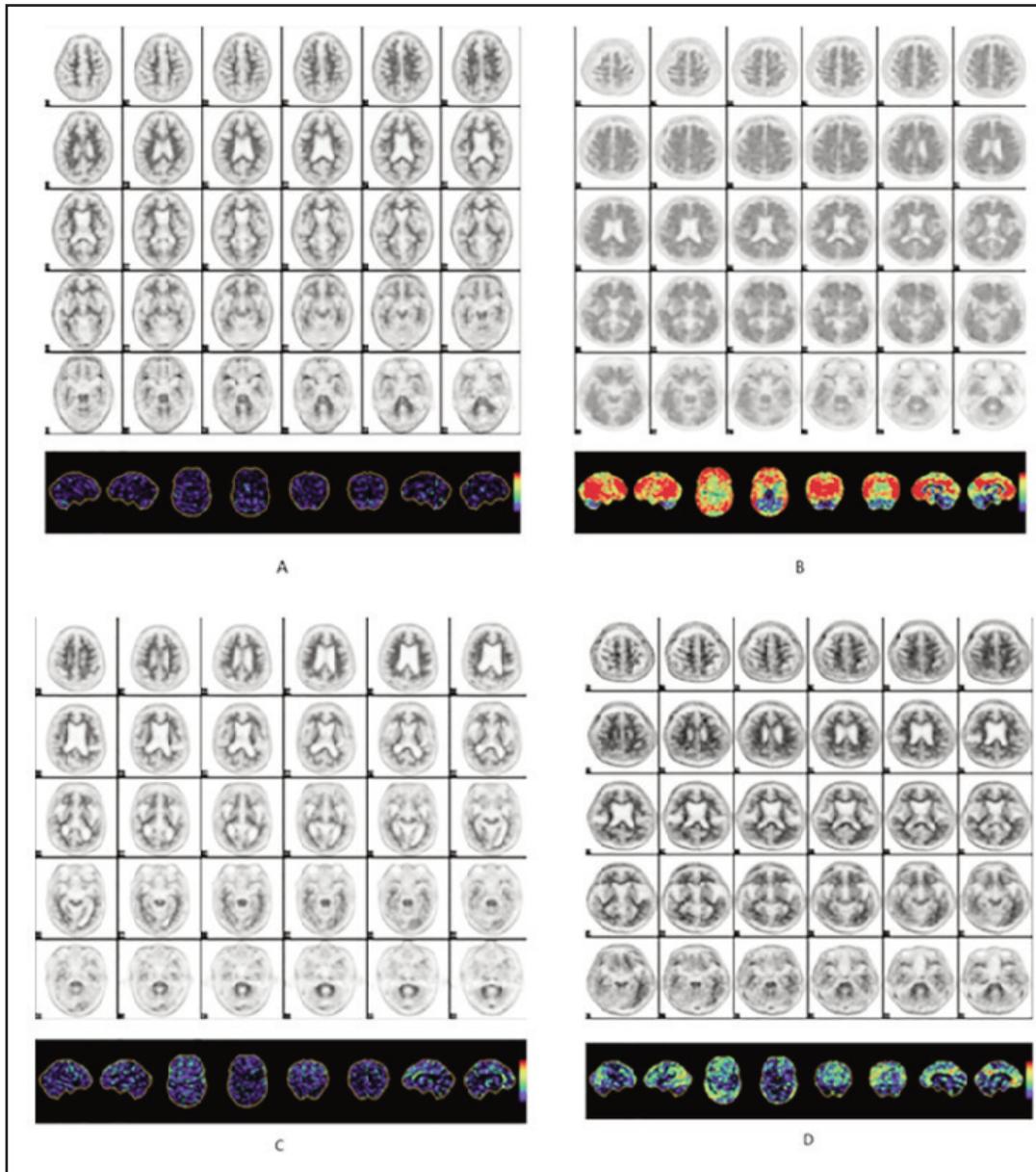
**Fig. 2:** Comparison of regional SUVRs and global cerebral cortex SUVRs from 18F-florbetapir PET (normalized by cerebellar cortex) of the clinical groups, showing overall higher SUVRs in AD than MCI and, in turn, than HC. Significant differences in the SUVRs were found between AD and MCI, and between AD and HC, but not between HC and MCI.



**Fig. 3:** Comparison of results of 18F-florbetapir brain PET from different interpretation methods, using the visual assessment and summary of stages obtained from 3D-SSP Z-score map patterns (the stages defined in Figure 1 were reclassified as follows: stages 0-I = negative, and stages II-IV = positive). The global cortical SUVR cutoff for amyloid positivity was > 1.15. A concordance of the results between the visual assessment and summed stages was observed, with HC > AD > MCI.

Significant negative correlations were partially demonstrated between both the global and regional SUVRs and the TMSE scores, with a medium strength of correlation ( $r = -0.341$  to  $-0.454$ ). In the MCI subgroup, significant correlations between

the amyloid depositions and the TMSE scores were observed at all regions other than the posterior cingulate and occipital regions. As to the AD subgroup, a significant correlation was found only between the occipital SUVR and the TMSE scores.



**Fig. 4:** Examples of trans-axial and 3D-SSP Z-score map images of <sup>18</sup>F-florbetapir PET. (A): A normal-cognitive, elderly individual with a clearly negative amyloid PET. (B): A moderate-AD patient with a clearly positive amyloid PET. (C): In equivocal cases, 3D-SSP imaging may be helpful in identifying the very early stage (stage I) of amyloid deposition in a patient with MCI and (D): A confirmed stage II amyloid deposition in a patient with clinically mild AD whose findings from the visual assessment were considered equivocal as the only focal abnormality was detected in the trans-axial images.

In this cohort, there was no significant correlation between the SUVRs and TMSE scores of the cognitively HCs (Table II).

**DISCUSSION**

The recommended interpretation criteria for amyloid PET/CT imaging based on visual assessment is simple. Nevertheless, this interpretation technique has the potential for variability among readers in equivocal cases as well as the limitation for assessing correlation with detailed neurocognitive performance. The limitations of subjective visual assessments may be overcome with additional, automated,

semiquantitative, analytical approaches; however, some of those require individual MRIs for anatomical co-registration. In the current study, we utilised a semiquantitative method of 3D-SSP Z-score mapping using freely accessible automatic software and a Thailand-specific NDB. We also drew on a recently proposed staging pattern<sup>18</sup> that provides an objective and more detailed interpretation of <sup>18</sup>F-florbetapir PET/CT imaging data than conventional binary interpretation. We expected that the Z-score mapping-based staging approach would improve the visual assessment of <sup>18</sup>F-florbetapir PET/CT imaging results, especially in equivocal cases. Furthermore, we expected that the staging approach may help to stratify

the severity of amyloid deposition and to identify minor longitudinal changes in regional amyloid deposition. These outcomes would be of benefit for clinical follow-up and the evaluation of the therapeutic efficacy of amyloid-clearing agents. We also expected to further demonstrate a correlation between the Z-score mapping-based stages and the degree of cognitive impairment.

In this study, we found a high association between the results of all the interpretation techniques of the amyloid PET/CT images and the clinical diagnoses, and a high concordance between the results interpreted by visual assessment and both semiquantitative approaches. However, equivocal cases may occur occasionally in real clinical practice, the examples are as shown in Figure 4. In our future study, we plan to investigate if the 3D-SSP technique has advantages over visual assessment by improving the level of diagnostic agreement and confidence in the interpretation of amyloid brain PET/CT images among readers with different experience levels and for diagnostic and prognostic values.

We found an overall higher SUVR in AD than in MCI, and in turn, than in HC. This is consistent with the results of previous studies, with analyses using either the 3D-SSP technique for amyloid PET/CT scans (without staging) or a radiotracer other than florbetapir,<sup>16</sup> or different analytical methods for <sup>18</sup>F-florbetapir PET/CT.<sup>30,31</sup> Nevertheless, significant differences in the SUVRs in our study were only found between AD and MCI, and between AD and HC, with no significant difference detected between the SUVRs of HC and MCI. In this cohort, the SUVR cutoff that best discriminated HC from AD (1.157) is slightly higher than the 1.1 recommended by previous studies.<sup>29,31,32</sup> This finding may support our hypothesis about the potential differences in amyloid PET/CT results among the population, although our cutoff is still within the range (1-1 – 1.34) used previously.<sup>30,33,34</sup>

There was no significant correlation between the TMSE scores and amyloid PET/CT using visual assessment. Although stronger correlations were demonstrated in MCI and AD with the Z-score map stages, they still did not meet statistical significance. Significant negative correlations between the TMSE scores and the SUVRs were partially demonstrated, with a medium strength of correlation; the MCI subgroup showed an overall stronger correlation and more regional brain involvement than the AD subgroup. These findings support our hypothesis that amyloid causes more negative neuropathological effects in the early stages rather than in the late stages of the disease. Our results support data from previous studies which found that the A $\beta$  burden correlated with disease severity and cognitive impairment at the preclinical and prodromal stages,<sup>35</sup> but not at the AD stage.<sup>36</sup> Interestingly, a significant correlation between the occipital SUVRs and TMSE scores was noted in AD. In the present cohort, there was no significant correlation between the SUVRs of amyloid PET/CT with the TMSE scores in HCs.

In a recent study, Mattsson et al.<sup>37</sup> proposed a staging system of A $\beta$  accumulation using a combination of CSF A $\beta$ 42 and <sup>18</sup>F-florbetapir PET/CT scan from ADNI data to evaluate the early, intermediate and late regions of A $\beta$  accumulation. The

early composite region in their study (precuneus, posterior cingulate, insula, medial and orbitofrontal cortices) overlaps the involved areas in Stages I–II in our study, while the late composite region (the lingual, pericalcarine, paracentral and postcentral cortices) also overlaps with Stages III–IV in our study. The ambiguous stage rate found in their staging system was 1.6%, which was similar to the 1.3% unstageable rate in our study. Their longitudinal study revealed an association between the higher stages and lower CSF A $\beta$ 42 concentrations, greater CSF P-tau and CSF T-tau and accelerated cognitive decline and brain atrophy. Therefore, they concluded that their staging system may be useful for monitoring the course of AD. The aspect of longitudinal change is also being explored in our ongoing study.

The lack of a strong correlation between amyloid accumulation in terms of the SUVRs and neurocognitive scores in our study supports the need for other biomarkers, e.g., <sup>18</sup>F-FDG PET/CT or Tau PET/CT, to identify the cause of cognitive decline. It also highlights the need for additional analysis of amyloid PET/CT results with more detailed neurocognitive scores representing different cognitive domains, which might be more sensitive for determining the correlation with corresponding regional brain changes in PET/CT. In our cohort, of the 6 patients with clinically probable AD whose amyloid PET/CT were negative by the Z-score mapping, four also showed negative amyloid PET/CT by visual assessment. The global cortical and regional SUVRs in these patients were within the range of the mean  $\pm$  SD of the HC subgroup. The additional <sup>18</sup>F-FDG PET/CT showed a normal study in 2 patients, an FTD pattern in 2 patients, a DLB pattern in 1 patient and a vascular change in 1 patient. These findings support the potential value of incorporating imaging biomarkers to improve the accuracy of diagnoses and the management of patients with dementia syndrome.<sup>38</sup> In 2 patients with MCI with a negative amyloid PET/CT but a positive <sup>18</sup>F-FDG PET/CT, indicating an early-to-mild AD pattern, these findings might be explained by either a neurodegenerative disease from a suspected non-Alzheimer's disease pathophysiology, or the degree of amyloid brain deposition is lower than the detectable threshold of PET/CT imaging. In contrast, 1 HC and 1 AD participant presented a positive amyloid PET/CT, but without any signs of neurodegeneration by either <sup>18</sup>F-FDG or MRI. It is known that positive cerebral amyloid deposition can be detected by PET/CT or autopsy in the cognitively normal elderly population.<sup>28</sup> However, long-term follow-up is currently underway to see if amyloid positivity can predict future changes in neurocognitive performance and the related neuroimaging findings. The detailed results from multimodal imaging including <sup>18</sup>F-florbetapir PET/CT, <sup>18</sup>F-FDG PET/CT and MRI together with the discordance between imaging findings and clinical diagnoses observed in the preliminary results mentioned above will be further clarified in our future publications. The ongoing research also aims to establish the longitudinal changes in, and the clinical significance of, the very early pattern of amyloid deposition (Stage I), which was detected in approximately 3% of our AD and MCI patients.

The main limitation of this study is the lack of a gold standard due to the unavailability of brain autopsy and CSF results to confirm the AD pathologies. A brain autopsy can

only be done in the post-mortem period, while a lumbar puncture for a CSF analysis is considered an invasive procedure. Diagnoses based on clinical criteria and neurocognitive tests alone are known to have limited accuracy, which might explain the relatively low amyloid positivity rate (up to 87%) by <sup>18</sup>F-florbetapir PET/CT in our AD subgroup. Moreover, there was an overlapping TMSE score range in the HC and MCI subgroups, which might explain the finding that there was no statistical difference in the TMSE scores of these subgroups. The relatively low range for the TMSE scores<sup>24-30</sup> in the criteria for the HC subgroup in our study was based on previous data for non-demented, elderly Thais, who had median TMSE scores of 27 (IQR 25–29) and 23 (IQR 19–26) for the literate and illiterate participants, respectively.<sup>39</sup> Therefore, to differentiate between the HC and MCI participants, we also used clinical complaints of cognitive impairment as well as other test scores in addition to the TMSE scores. With this limitation, we did not focus on comparing the diagnostic performance of amyloid PET between visual and semiquantitative techniques (staging by Z-score map pattern and SUVR) in this initial study; however, the reference tests used in our cross-sectional study to identify the patient subgroups were similar to those of other studies.<sup>11,28,31,32</sup> A longitudinal study on changes in neurocognitive performance and neuroimaging findings in this cohort is still being performed to evaluate if any parameters may serve as prognostic indicators of neurocognitive decline. Another limitation is that the 3D-SSP FDG-amyloid technique needs <sup>18</sup>F-FDG PET/CT to co-register <sup>18</sup>F-florbetapir PET/CT to the standard brain template, which leads to concerns pertaining to additional patient radiation dose, time and cost of the study, although the benefit of incorporating results from FDG PET may outweigh this limitation. On the one hand, the co-registration of <sup>18</sup>F-florbetapir PET/CT to the standard template without the use of a structural MRI scan can be considered to be an advantage, especially in patients for whom an MRI cannot be performed. A new version of 3D-SSP analysis for amyloid PET/CT without the need for <sup>18</sup>F-FDG PET/CT is being developed and validated to overcome these limitations. Despite all the 3D-SSP limitations mentioned, this technique offers the ability to objectively evaluate cerebral amyloid deposition using a fully automated, PET/CT-based approach, with operator convenience and independence, a saving of time, and economic effectiveness.

## CONCLUSION

The 3D-SSP analysis of <sup>18</sup>F-florbetapir PET/CT images enabled a fully automated, semiquantitative analysis of cerebral amyloid deposition using a normal database specific for our population. The technique provided objective patterns of amyloid distribution in the brain and semiquantitative results that were associated with the diagnosis and neurocognitive performances in MCI and AD patients.

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