Association Between In Vivo Fluorine 18–Labeled Flutemetamol Amyloid Positron Emission Tomography Imaging and In Vivo Cerebral Cortical Histopathology

David A. Wolk, MD; Igor D. Grachev, MD, PhD; Chris Buckley, PhD; Hala Kazi, PhD; M. Sean Grady, MD; John Q. Trojanowski, MD, PhD; Roy H. Hamilton, MD; Paul Sherwin, MD, PhD; Richard McLain, MS; Steven E. Arnold, MD

Objective: To determine the correspondence of in vivo quantitative estimates of brain uptake of fluorine 18–labeled flutemetamol with immunohistochemical estimates of amyloid levels in patients who underwent previous biopsy.

Design: Cross-sectional study of 18F-flutemetamol positron emission tomography (PET) findings in patients with prior cortical biopsy specimen stained for the presence or absence of amyloid plaques.

Setting: University hospital.

Patients: Seven patients who previously had a prior right frontal cortical biopsy at the site of ventriculoperitoneal placement for presumed normal pressure hydrocephalus were recruited. Inclusion criteria included an adequate biopsy specimen for detection and quantification of β-amyloid pathology and age older than 50 years.

Intervention: All patients underwent an 18F-flutemetamol PET scan.

Main Outcome Measures: Quantitative measures of 18F-flutemetamol uptake (standardized uptake value ratio, a ratio of mean target cortex activity divided by that in a cerebellar reference region) were made at a location contralateral to the biopsy site and compared with estimates of amyloid load based on immunohistochemical and histological staining.

Results: There was complete agreement between visual reads of 18F-flutemetamol PET scans (3 blinded readers with majority rule) and histology. A regression model, including time from biopsy as a covariate, demonstrated a significant relationship (P = .01) between 18F-flutemetamol uptake and percentage of area of amyloid measured by a monoclonal antibody raised against amyloid (NAB228). Similar results were found with the amyloid-specific monoclonal antibody 4G8 and Thioflavin S.

Conclusion: To our knowledge, these data are the first to demonstrate the concordance of 18F-flutemetamol PET imaging with histopathology, supporting its sensitivity to detect amyloid and potential use in the study and detection of Alzheimer disease.


THE DEVELOPMENT OF MOLECULAR imaging techniques to visualize the pathology of Alzheimer disease (AD) in vivo represents a major achievement for the diagnosis and study of neurodegenerative conditions. In addition to a potential role in early and accurate diagnosis in clinical practice, molecular imaging is likely to play a critical role in drug development through cohort enrichment by increasing diagnostic confidence, clarification of drug mechanism and dosage, and assessment of biological effect. Over the last decade, several positron emission tomography (PET) radioligands have displayed affinity for fibrillar β-amyloid (Aβ) aggregates, one of the hallmark pathologic lesions of AD.

See also pages 1377 and 1404

CME available online at www.jamaarchivescme.com and questions on page 1373

To date, carbon 11–labeled Pittsburgh Compound B (11C-PiB) (2-[4-methylamino phenyl]-1, 3-benzothiazol-6-ol) is the most well studied of these PET amy-
loid imaging ligands. Pittsburgh Compound B is a neutral analog of Thioflavin T, an established histological stain for detecting Ab. 11C-Pittsburgh Compound B has demonstrated success in discriminating patients with probable AD from healthy controls and predicting the likelihood of clinical progression in those with mild cognitive impairment to frank AD. Additionally, 11C-PiB uptake inversely correlates with levels of Ab42 in the cerebrospinal fluid and Ab aggregates in a limited number of postmortem and biopsy studies. Despite the considerable promise of 11C-PiB, its potential for widespread use is severely limited by the short half-life of 11C (about 20 minutes), which requires on-site cyclotron production.

The radionuclide fluorine 18 has a longer half-life (about 110 minutes), allowing for shipment to scanning sites after production, and has been successfully used in other PET tracers (eg, fluorodeoxyglucose). Several 18F-based amyloid PET imaging ligands with divergent structural properties from 11C-PiB are at various stages of development, including recent work demonstrating a high concordance between 18F-florbetapir and postmortem histology. Alternatively, the novel ligand 18F-flutemetamol only differs from 11C-PiB with regard to the radionuclide. Recent work has suggested that 18F-flutemetamol demonstrates similar imaging findings in patients with probable AD and mild cognitive impairment relative to healthy controls and has a high correlation with 11C-PiB when tested in the same patients. However, histopathological confirmation of the in vivo findings of this compound are lacking.

Normal pressure hydrocephalus (NPH) is a progressive condition associated with a classic triad of dementia, gait abnormalities, and urinary incontinence. Despite its apparent distinctiveness, this condition can be difficult to diagnose and the presence of AD pathology may alter response to ventricular shunting. Prior work has suggested that a significant proportion of patients with NPH demonstrate AD pathological lesions on biopsy specimen. Indeed, in a prior series, we found that approximately 68% of patients with NPH had evidence of AD pathology in a small cortical biopsy specimen obtained at the site of ventriculoperitoneal shunt placement and that higher levels of AD pathology were associated with poorer response to the surgery. Thus, amyloid imaging may be of diagnostic and prognostic value in such patients. In the current study, we leveraged pathologic data available from these patients with NPH who underwent previous biopsy to assess concordance with in vivo 18F-flutemetamol PET imaging.

**METHODS**

**PARTICIPANTS**

Patients were recruited from the Penn Memory Center who had undergone ventriculoperitoneal shunting with concomitant biopsy. The majority participated in a previously reported longitudinal study of the impact of AD pathology on clinical response to shunting. In addition to a biopsy that was adequate for detection and quantification of Ab pathology, inclusion criteria in-
liquid chromatography. The radiopharmacist at the PET imaging site ensured that the correct activity was present in the injection syringe and that the product was used within the validity period. Subjects received $^{18}$F-flutemetamol injection under the direct supervision of study personnel. Each subject received an intravenous dose of $^{18}$F-flutemetamol injection (<10 µg of total $^{18}$F-flutemetamol). The activity of a single administration of $^{18}$F-flutemetamol injection was approximately 185 MBq (range, 111 MBq-197 MBq).

Dynamic brain scanning was performed on an Allegro whole-body PET scanner (Philips, Andover, Massachusetts). The duration of the PET imaging was 30 minutes beginning approximately 90 minutes after the administration of $^{18}$F-flutemetamol injection. Dynamic PET scans of the whole brain were obtained in a single field of view. The dynamic PET data consisted of six 5-minute frames.

$^{18}$F-FLUTEMETAMOL PET VISUAL IMAGE ANALYSIS

Blinded visual reads were performed by 3 trained raters, 2 nuclear physicians and a senior medical physicist, with experience reading amyloid scans to determine an abnormal or normal scan for increased $^{18}$F-flutemetamol uptake. Readers worked independently and visually assessed the level of $^{18}$F-flutemetamol uptake in cortical regions with reference to the uptake in the cerebellum. The review method was to locate a sagittal view plane along the longitudinal fissure and move a few millimeters from the center until the view plane encountered the medial gyri and sulci. Having completed one hemisphere, the opposite hemisphere was surveyed in the same manner. An axial review was then carried out working in the dorsal to ventral direction.

If readers found elevated cortical uptake in at least 1 region, with emphasis on frontal/anterior cinguli, posterior cinguli/precuneus, and lateral temporal regions, the image would be rated as abnormal. Otherwise the image was rated as normal. In cases of disagreement, assignment was based on the majority verdict.

$^{18}$F-FLUTEMETAMOL PET QUANTITATIVE IMAGE ANALYSIS

From the dynamic PET data, a 30-minute summed PET image was derived. The reconstruction method was a 3-dimensional row-action maximum likelihood algorithm, giving an approximate spatial resolution of 6 mm. The blinded visual assessment was based on the summed images in native space. For quantitative analysis, this summed PET image was coregistered with the subject's magnetic resonance imaging to define volumes of interest (VOIs). Two types of target VOIs were determined in the following manner: (1) a hollow VOI surrounding the excised tissue area (representing the most adjacent sampling achievable) and (2) a VOI sampling the same anatomical region on the contralateral side. Use of a contralateral site is based on prior work demonstrating only small differences in PiB uptake for symmetrically placed VOIs in the left and right frontal cortices. Publication Figure 1 shows the location of the biopsy in a $^{18}$F-flutemetamol multiplanar reconstruction view. A composite VOI was also calculated as the mean of several anatomic regions typically associated with significant amyloid plaque burden in AD (ie, frontal cortex, anterior cingulate gyrus, posterior cingulate gyrus/precuneus, lateral-temporal cortex, and parietal cortex), as previously described.

Standardized uptake value ratios (SUVRs), defined as $\text{SUVR}_{\text{VOI}} / \text{SUVR}_{\text{REF}}$ with SUV being the integrated activity over a given period per unit of injected dose and body weight, were calculated for the VOIs. The reference region used for this study was the cerebellar cortex. Thepons was also used as a reference region to explore if the SUVR vs the percentage of area comparisons would show a higher degree of correlation. No significant improvement was observed and, as a result, these data were not included.

**STATISTICAL ANALYSIS**

To determine the relationship between $^{18}$F-flutemetamol uptake and amyloid plaque burden on biopsy, both dichotomous (presence or absence) and continuous variable statistics were used. A qualitative read of scans classified them as abnormal or normal and the microscopic inspection of tissue sections classified them as normal (no Aβ plaques) or abnormal (Aβ plaques present). For continuous variable analyses, regression models included percentage of area of Aβ plaque as the dependent variable while the PET SUVR measure for the VOI was the independent variable. A covariate for the interval from biopsy to PET scan was included. All statistics were performed with SAS version 9.2 (IBM SAS, Chicago, Illinois) and were prespecified in the study protocol statistical analysis plan.

**RESULTS**

Demographic data are presented in the Table. Four patients displayed evidence of amyloid plaque pathology based on NAB228 and 4G8 immunohistochemical and Thioflavin S histological staining (Figure 2). Corresponding $^{18}$F-flutemetamol PET scans are also displayed.

There was high concordance for the visual reads across the 3 raters, with disagreement on only 1 scan (this scan was rated as abnormal by 2 of the 3 raters). By majority verdict, 4 of 7 scans were considered abnormal. In each of these cases, the biopsy tissue contained amyloid plaque pathology. The 3 normal $^{18}$F-flutemetamol scans were all in patients without evidence of Aβ plaque pathology.

The regression model for NAB228 was statistically significant ($F = 10.1; P < .05; \beta = 0.63; 95\% \text{ confidence interval}, 0.24-1.02$), and there was no apparent modulating influence of the interval from biopsy to the PET scan ($P > .21$). The relationship between these measures can be seen in Figure 3. Similar results were obtained when percentage of area

**Table**: Demographic data are presented in the Table. Four patients displayed evidence of amyloid plaque pathology based on NAB228 and 4G8 immunohistochemical and Thioflavin S histological staining (Figure 2). Corresponding $^{18}$F-flutemetamol PET scans are also displayed.
estimates of Aβ were determined with 4G8 (model: $F = 10.0; P < .05; R^2 = 0.83; \beta = 2.00$; 95% confidence interval, 0.76-3.24) and Thioflavin S (model: $F = 15.6; P < .05; R^2 = 0.89; \beta = 0.56$; 95% confidence interval, 0.28-0.84). Ipsilateral VOI SUVRs produced similar findings for 4G8 and Thioflavin S (both $P < .05$), although the regression model with percentage of area of NAB228 staining did not reach significance ($P = .08$). The composite VOI also produced similar results and is not presented herein for brevity.

Single doses of 18F-flutemetamol were well tolerated. Five subjects (71.4%) experienced a total of 8 adverse events. The majority of the adverse events reported were mild in intensity. Six (in 4 subjects) were considered to be possibly related or related to 18F-flutemetamol injection. The most frequently reported adverse event was increase in blood pressure (5 subjects [71.4%] with 5 adverse events). The remaining 3 adverse events (dizziness, blood glucose level decreased, and flushing) all occurred in 1 subject. The mild to moderate increases of blood pressure observed in 3 subjects resolved without treatment and were considered related to the administration of 18F-flutemetamol injection. No deaths, serious adverse events, or withdrawals due to adverse events occurred during the study. No clinically significant abnormalities were noted in clinical laboratory results. There were no clinically significant changes in electrocardiograph results.

To our knowledge, the present data represent the first study to compare the novel in vivo amyloid imaging ligand 18F-flutemetamol with in vivo histopathological evidence of AD-related amyloid pathology. Despite vari-

**Table. Patient Characteristics**

<table>
<thead>
<tr>
<th>Patient/Sex/Age, y</th>
<th>Education, y</th>
<th>MMSE Score</th>
<th>Time From Biopsy, mo</th>
<th>Amyloid Plaques on Biopsy Specimen</th>
<th>Composite SUVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/78</td>
<td>16</td>
<td>28</td>
<td>33</td>
<td>+</td>
<td>2.13</td>
</tr>
<tr>
<td>2/F/75</td>
<td>12</td>
<td>23</td>
<td>14</td>
<td>+</td>
<td>2.48</td>
</tr>
<tr>
<td>3/M/82</td>
<td>12</td>
<td>23</td>
<td>26</td>
<td>−</td>
<td>1.39</td>
</tr>
<tr>
<td>4/F/75</td>
<td>12</td>
<td>17</td>
<td>22</td>
<td>+</td>
<td>2.74</td>
</tr>
<tr>
<td>5/F/66</td>
<td>12</td>
<td>29</td>
<td>4</td>
<td>−</td>
<td>1.34</td>
</tr>
<tr>
<td>6/M/66</td>
<td>12</td>
<td>27</td>
<td>−</td>
<td></td>
<td>1.34</td>
</tr>
<tr>
<td>Mean</td>
<td>12.4</td>
<td>23.0</td>
<td>26.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MMSE, Mini-Mental State Examination; SUVR, standardized uptake value ratio; +, present; −, absent.

*Mean age, 70.4 years.*

**Figure 2.** Representative NAB228-stained tissue is displayed for each of the 7 patients in this study. Above each is an axial image of the corresponding fluorine 18-labeled flutemetamol positron emission tomography scan. Scans from patients 1, 2, 4, and 5 were classified as abnormal by the independent readers, while scans from patients 3, 6, and 7 were classified as normal. These designations corresponded to ratings of the pathology. Color-scale reference represents zero to maximum activity concentration (megabecquerel per milliliter) in each image. The scale bars in the photomicrographs are 200 µm.

**Figure 3.** Relationship of estimated Aβ-amyloid (Aβ) deposition in the biopsy sample (percentage of area of NAB228 monoclonal antibody) and fluorine 18-labeled flutemetamol positron emission tomography uptake in the contralateral volume of interest (VOI). SUVR indicates standardized uptake value ratio.
ability in the delay from biopsy to PET scanning, there was a remarkable correspondence of $^{18}$F-flutemetamol uptake and quantitative measures of amyloid pathology based on immunohistochemical and histological estimates. Additionally, masked visual assessments of these scans were consonant with the histopathology results. These findings are supportive of the potential role of $^{18}$F-flutemetamol PET imaging in the detection of fibrillar $\alpha$B plaques. As with $^{11}$C-PiB, binding is expected to be more prominent to neuritic than diffuse plaques.16

The current results are consistent with the still relatively limited, but accruing, histopathological data for $^{11}$C-PiB PET imaging.14-17,32,33 For example, Ikonomovic and colleagues39 reported regional correlations between in vivo $^{11}$C-PiB uptake and quantitative measures of $\alpha$B pathology in a patient who had autopsy performed 10 months after PET imaging. Of most direct relevance to this report is a study in which right frontal cortical biopsy specimens were obtained in 10 patients being evaluated for suspected NPH with intracranial pressure monitoring.17 Patients underwent $^{11}$C-PiB scanning 2 to 36 months after biopsy. Overall, there was a reasonably high correlation of $^{11}$C-PiB binding in the right frontal cortex and the number of $\alpha$B aggregates on biopsy specimen. Of the 5 cases without clear evidence of fibrillar amyloid pathology, none displayed elevated $^{11}$C-PiB uptake. Alternatively, 4 of 5 cases with evidence of $\alpha$B pathology had a clearly abnormal $^{11}$C-PiB scan, but there was 1 false-negative result. An additional false-negative $^{11}$C-PiB result was recently reported in a patient whose scan was performed 2½ years prior to autopsy.31 The explanation for these normal scans in the context of $\alpha$B pathology remains unclear, but one potential explanation is that conformational variants of the secondary or tertiary structure of $\alpha$B may influence PiB binding. Prolonged timing between imaging and autopsy may also contribute because of possible disease progression.34 To date, there have been no reports of false-positive $^{11}$C-PiB results based on histopathology.

Similar to this work with $^{11}$C-PiB, $^{18}$F-flutemetamol uptake also showed a strong association with quantitative measures of amyloid burden. This was the case for both a region surrounding the right frontal biopsy site and an analogous VOI in the contralateral hemisphere, consistent with the view that there generally is little asymmetry in amyloid pathology.31 These correlations are perhaps somewhat surprising given the substantial delay in the time from biopsy to the PET scan in several cases. However, this result is commensurate with the relatively slow change in amyloid levels that has been observed in prior $^{11}$C-PiB PET longitudinal studies.35-37 Possibly of greatest relevance to potential clinical applicability of $^{18}$F-flutemetamol PET scans was the accuracy of clinical reads relative to the presence or absence of $\alpha$B pathology on the biopsy specimens. Such dichotomous visual reads are likely the strategy that will be used in clinical practice, as with other imaging modalities.

Single doses of $^{18}$F-flutemetamol were well tolerated in subjects with NPH. The mechanism and possible explanation of mild to moderate increases of blood pressure observed in 3 subjects, which resolved without treatment, is unknown and may be related to the patient reaction to study procedures (eg, intravenous catheter placement, blood draws).

The current study has a number of limitations. Sample size was modest. The small cortical biopsy specimen could have led to sampling error with presence of AD pathology in other brain regions despite an unremarkable biopsy result. Additionally, the delay between the time from biopsy to PET scanning may have been associated with some patients developing AD pathology during that interval. The latter 2 issues would seemingly have resulted in false-positive scans relative to the biopsy findings, which would have presented interpretative difficulties. Nonetheless, there were no instances of such a result. As noted earlier, the lag between scan and biopsy also may have reduced the association between quantitative $^{18}$F-flutemetamol uptake and biopsy-determined amyloid load, particularly given differences in this delay across subjects and the potential for different rates of accumulation.

These findings provide support for the use of $^{18}$F-flutemetamol PET in the detection of AD-related amyloid pathology. As has been suggested by work with $^{11}$C-PiB PET, this information may play an important prognostic role in patients with mild cognitive impairment and, perhaps, in preclinical populations.10,38,39 With the potential emergence of disease-specific interventions for AD, biomarkers that provide molecular specificity will likely become of greater importance in the differential diagnosis of cognitive impairment in older adults. Furthermore, amyloid imaging may serve an important role in the development of such interventions by allowing for assessment of biological effect.40 Finally, amyloid imaging agents such as $^{18}$F-flutemetamol may be of particular relevance to the NPH population studied herein given evidence that the presence of AD pathology in patients with suspected NPH may be associated with a poorer outcome after ventricular shunt placement, which may impact decisions on treatment.23 For example, an abnormal scan may lead to a clinical decision to avoid the expense and potential medical complications associated with shunt placement in a patient unlikely to obtain a significant benefit from the procedure. All of these potential roles for amyloid imaging agents will be greatly facilitated by the increased availability of $^{18}$F PET radioligands.

Accepted for Publication: April 28, 2011.


Author Affiliations: Penn Memory Center (Drs Wolk, Hamilton, and Arnold), Departments of Neurology (Drs Wolk and Hamilton), Psychiatry (Drs Kazi and Arnold), Neurosurgery (Dr Grady), and Pathology (Dr Trojanowski), Institute on Aging (Dr Trojanowski), and Center for Neurodegenerative Disease Research (Dr Trojanowski), University of Pennsylvania, Philadelphia; Clinical Development, Medical Diagnostics, GE Healthcare, Princeton, New Jersey (Drs Grachev and Sherwin); Imaging Technology, Medical Diagnostics, GE Healthcare, Amersham, England (Dr Buckley); and FP Statistical Consulting, LLC, Livonia, Michigan (Mr McLain).

Correspondence: David A. Wolk, MD, Penn Memory Center, 3615 Chestnut St, Philadelphia, PA 19104 (d.wolk@uphs.upenn.edu).

Author Contributions: Study concept and design: Wolk, Grachev, Trojanowski, Sherwin, and McLain. Acquisition of data: Wolk, Buckley, Kazi, Grady, Trojanowski,
Financial Disclosure: The study was entirely sponsored by GE Healthcare. Dr Wolk has received consulting fees from GE Healthcare. Drs Grachev, Buckely, and Sherwin are employees of GE Healthcare. Mr McLain is a contractor for GE Healthcare.

Additional Contributions: We acknowledge the staff of i3 Statprobe for biometric services, programming, and statistical analyses. We thank GE Healthcare study team members for operational support (Kim Mansfield, MS), project management (Gill Farrar, PhD), data management (Ginger Lewis, MBA), and medical writing assistance with the study report (Ann Tate, MS, and Chris Devine, BSc).

REFERENCES