Cerebral PET with florbetapir compared with neuropathology at autopsy for detection of neuritic amyloid-β plaques: a prospective cohort study

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Summary

Background **Results of previous studies have shown associations between PET imaging of amyloid plaques and amyloid-β pathology measured at autopsy. However, these studies were small and not designed to prospectively measure sensitivity or specificity of amyloid PET imaging against a reference standard. We therefore prospectively compared the sensitivity and specificity of amyloid PET imaging with neuropathology at autopsy.**

Methods **This study was an extension of our previous imaging-to-autopsy study of participants recruited at 22 centres in the USA who had a life expectancy of less than 6 months at enrolment. Participants had autopsy within 2 years of PET imaging with florbetapir (^{18}F). For one of the primary analyses, the interpretation of the florbetapir scans (majority interpretation of five nuclear medicine physicians, who classified each scan as amyloid positive or amyloid negative) was compared with amyloid pathology (assessed according to the Consortium to Establish a Registry for Alzheimer's Disease standards, and classed as amyloid positive for moderate or frequent plaques or amyloid negative for no or sparse plaques); correlation of the image analysis results with amyloid burden was tested as a coprimary endpoint. Correlation, sensitivity, and specificity analyses were also done in the subset of participants who had autopsy within 1 year of imaging as secondary endpoints. The study is registered with ClinicalTrials.gov, number NCT 01447719 (original study NCT 00857415).**

Findings **We included 59 participants (aged 47–103 years; cognitive status ranging from normal to advanced dementia). The sensitivity and specificity of florbetapir PET imaging for detection of moderate to frequent plaques were 92% (36 of 39; 95% CI 78–98) and 100% (20 of 20; 80–100%), respectively, in people who had autopsy within 2 years of PET imaging, and 96% (27 of 28; 80–100%) and 100% (18 of 18; 78–100%), respectively, for those who had autopsy within 1 year. Amyloid assessed semiquantitatively with florbetapir PET was correlated with the post-mortem amyloid burden in the participants who had an autopsy within 2 years (Spearman ρ=0·76; p<0·0001) and within 12 months between imaging and autopsy (0·79; p<0·0001).**

Interpretation **The results of this study validate the binary visual reading method approved in the USA for clinical use with florbetapir and suggest that florbetapir could be used to distinguish individuals with no or sparse amyloid plaques from those with moderate to frequent plaques. Additional research is needed to understand the prognostic implications of moderate to frequent plaque density.**

Funding **Avid Radiopharmaceuticals.**

Introduction

PET imaging biomarkers such as Pittsburgh compound B (^{11}C-PIB),[1] florbetaben (^{18}F),[2] flutemetamol (^{18}F),[3] and florbetapir (^{18}F)[4] provide the potential for in-vivo identification of amyloid-β pathology in patients being assessed for Alzheimer's disease or other causes of cognitive decline. We have shown a strong relation between amyloid-β density estimated from florbetapir PET imaging (with semiquantitative visual interpretation and quantitative standard uptake value ratio [SUVR]) and that assessed by use of immunohistochemistry or silver stain at autopsy.[5] Results of other studies with ^{11}C-PIB and flutemetamol have similarly shown associations between amyloid burden estimated by use of PET imaging and amyloid levels at autopsy or after biopsy.[6–10] However, so far, these studies have all been small and their results have not established the sensitivity or specificity of these PET tracers compared with a defined pathology reference standard.

Our previous study[5] was designed to continue until 35 autopsy assessments had been obtained. The current study is a continuation of the previous one. Participants who were alive and therefore did not have an autopsy in the original study were followed up to autopsy or for an additional year (maximum of 2 years) after the PET scan. Images and histopathological results from the original and extended follow-up were analysed together to test the sensitivity and specificity of a binary visual interpretation (ie, amyloid positive or amyloid negative) of florbetapir PET scans by comparison with the reference standard of neuritic plaque density at autopsy. We also aimed to confirm, in this larger population, the correlation

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See Online for appendix

previously noted between the PET scan and a quantitative immunohistochemical assessment of brain amyloid β.

Methods

Participants

Participants with a cognitive status of normal to advanced dementia were enrolled at 22 centres across the USA between February, 2009, and March, 2010, from inpatient and community hospice centres, long-term-care facilities, and outpatient community health-care and memory centres as part of our previous study.[5] All participants had a life expectancy of less than 6 months in the opinion of the enrolling physician at entry and no evidence of destructive brain lesions that would interfere with interpretation of the PET scan. All patients who met the enrolment criteria were included. Data from all 59 participants who had an autopsy were included in this analysis.

All participants or their designated decision maker provided written informed consent. The study was approved by the relevant institutional review boards and done in accordance with the guidelines of the International Conference on Harmonisation for Good Clinical Practice.

Procedures

Acquisition of florbetapir PET scan images was done at 20 imaging centres as has been described previously.[5] Briefly, a 10 min PET acquisition was done about 50 min after administration of 370 MBq (10 mCi) florbetapir. Images were acquired with a 128×128 matrix and reconstructed with iterative or row action maximisation likelihood algorithms with post-reconstruction Gaussian filter.

Images were interpreted with two different methods by two sets of readers (five in the sensitivity and specificity study and three in the correlation analysis). For measurement of sensitivity and specificity of the florbetapir PET scan, five board-certified nuclear medicine physicians were selected. Two had previous experience in amyloid imaging. Readers, masked to all clinical and neuropathological data, were trained to do binary classification of scans as either amyloid positive (increased retention of tracer in cortical grey matter as shown by the apparent loss of contrast in grey or white matter in any two cortical regions or intense uptake in at least one cortical region) or amyloid negative (low retention of tracer in cortical grey matter), in accordance with the methods proposed for clinical use of the tracer. A nuclear medicine physician with expertise in amyloid imaging (MAM) presented an overview of the binary interpretation method followed by practice cases for discussion. All training and study images were reviewed in native space, mainly in transaxial orientation, with sagittal and coronal planes accessible if needed, using a black and white palette with maximum intensity of the scale set to the maximum intensity brain pixel. All training images were from patients from studies other than the current study. After completion of training, scans from the autopsy test participants were presented independently in the same (random) order to each of the five readers, and the classification given by the majority of readers was used as the primary imaging outcome variable for sensitivity and specificity.

For **methods proposed for clinical use** see http://pi.lilly.com/us/amyvid-uspi.pdf

Assessments of images by use of a semiquantitative visual analysis (rated from 0 [no tracer retention in cortical grey matter] to 4 [high levels of cortical amyloid-β deposition]) had been done by a different set of readers, all newly trained in amyloid imaging, as part of the initial enrolment study,[5] and their median semiquantitative rating for each of the 59 scans from participants who had autopsy was the primary outcome variable for correlation analysis.

In addition to the visual readings of scan images, semiautomated quantitative analysis (cortical to cerebellar SUVR) was done using the mean signal of six predefined anatomically relevant cortical regions of interest (frontal, temporal, parietal, precuneus, anterior cingulate, and posterior cingulate) with the whole cerebellum used as a reference region. A threshold for positivity was set at greater than 1·10, based on the upper 95% CI of SUVR in a control sample of young healthy participants in a previous study by use of identical analytical methods.[11]

Methods for detection and quantification of amyloid β in brain sections at autopsy were similar to those previously described.[5] Because immunohistochemistry does not have a recognised cutoff that can be used as a positive neuropathological finding, neuritic plaque densities were used instead for the sensitivity and specificity analyses. Neuritic plaques were labelled using a modified Bielschowsky[12] silver stain and plaque density in the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) diagnostic regions (middle frontal gyrus, superior and middle temporal gyri, and inferior parietal lobe) was scored by two independent experienced neuropathology raters and reviewed by a senior neuropathologist (JAS), with no access to clinical or imaging information. The mean rater estimate was converted to a semiquantitative rating (1=none, 2=sparse, 3=moderate, or 4=frequent neuritic plaques) using modified CERAD criteria.[13] This classification was then converted to a binary scale, with absent or sparse neuritic plaques classified as amyloid negative, and moderate or frequent as amyloid positive. This binary classification of the density of neuritic amyloid plaques was selected as the main reference standard for measurement of sensitivity and specificity of florbetapir PET scans, based on guideline recommendations that moderate or frequent plaques, in the presence of threshold neurofibrillary tangle density and distributions, support a pathological diagnosis of at least probable Alzheimer's disease (CERAD) or intermediate likelihood Alzheimer's disease neuropathological change (National Institute on

Aging [NIA]-Reagan and NIA-Alzheimer's Association), and are required for a neuropathological diagnosis of definite Alzheimer's disease (CERAD) or high likelihood Alzheimer's disease (NIA-Reagan and NIA-Alzheimer's Association).[13–15]

Immunohistochemistry with the 4G8 anti-amyloid-β monoclonal antibody (Covance, Emeryville, CA, USA) was used to quantify amyloid-β density in tissue sections in six cortical regions from both hemispheres (frontal, temporal, parietal, precuneus, anterior cingulate, and posterior cingulate).[5] The neuropathological measure was the mean amyloid-β burden in the six cortical regions (percentage of grey matter area labelled by immunohistochemistry).

Statistical analysis

The primary and secondary efficacy analysis populations were all participants who had autopsy within 24 months and 12 months of their florbetapir PET scan, respectively. Two primary analyses were done in the study.

First, sensitivity and specificity of the results of the binary (qualitative) PET imaging were calculated by use of the binary assessment of the presence or absence of significant (more than sparse) neuritic amyloid plaques at autopsy as the reference standard. The primary hypothesis for this analysis was that the sensitivity and specificity of florbetapir PET would be greater than 80%.

The second primary analysis was the evaluation of the correlation between the semiquantitative visual assessment of the PET scan and mean amyloid-β density in the six target cortical regions, as measured with immunohistochemistry at autopsy, with the primary hypothesis that there was a significant relation (null hypothesis: $\rho=0$).

Secondary analyses were the same as the primary analyses, except that they were undertaken on the subset of patients who had autopsy within 12 months of the florbetapir PET scan. Other analyses were exploratory and included additional correlation analyses (eg, between the semiquantitative visual reading of PET scans and modified CERAD score or between SUVR and mean amyloid-β density as measured with immunohistochemistry at autopsy), sensitivity and specificity analyses by individual readers, and inter-reader agreement ratings.

A type 1 error rate of 5% was set for all analyses and no adjustments were planned for multiple comparisons in the exploratory analyses. The type 1 error was set as one-sided for correlation analyses and as two-sided for all other analyses. 95% CIs were calculated for sensitivity, specificity, and accuracy with the Wilson score method. The Spearman rank correlation coefficient was used to assess the relation and 95% CIs were obtained with Fisher z transformation. A simple κ statistic was used to assess the pairwise inter-reader agreement and a Fleiss' κ was used to assess the inter-reader agreement between all five readers. All statistical analyses were done with SAS (version 8.1 or higher).

The study is registered with ClinicalTrials.gov, number NCT 01447719 (original study NCT 00857415).

Role of the funding source

The study protocol was designed by the sponsor (Avid Radiopharmaceuticals) in consultation with the investigators who are named as authors and the study was initiated by the sponsor together with the investigators. The sponsor supported the collection, analysis, and interpretation of the data, and was involved in the writing of the report. The corresponding author had full access to all the data in this study and had final responsibility for the decision to submit for publication. All the essential data supporting the results and conclusions in this study were available to all authors.

Results

152 participants (aged 47–103 years) were recruited in the current study.[5] In total, 147 valid scans were obtained. Figure 1 shows the flow of individuals in the current study. Five participants were excluded because of invalid (poor-quality) scans (from excessive movement or camera failure). The initial study reached its predefined stopping point after 35 participants died and had an autopsy. The remaining participants were followed up for 1 year thereafter, or to a maximum of 2 years after the original

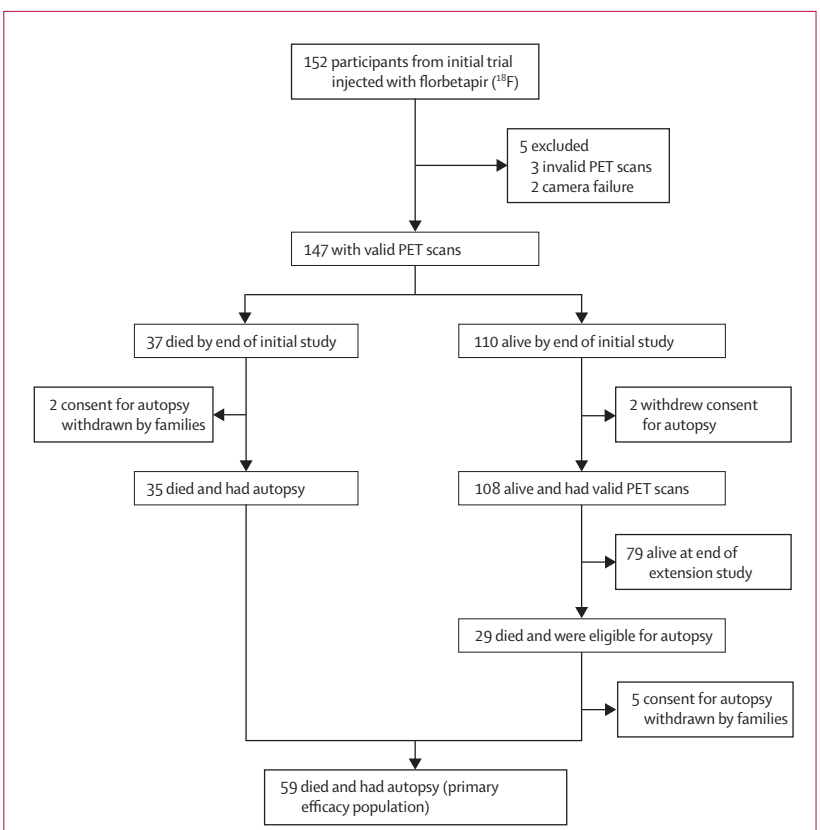

Figure 1: **Study profile**

	≤12 months autopsy cohort (n=46)	≤24 months autopsy cohort (n=59)
Age at enrolment (years)	79·0 (12·38)	79·4 (12·64)
Women	21 (46%)	29 (49%)
Race		
White	42 (91%)	55 (93%)
Black	3 (7%)	3 (5%)
Other	1 (2%)	1 (2%)
Ethnic origin		
Not Hispanic or Latino	42 (91%)	54 (92%)
Hispanic or Latino	4 (9%)	5 (8%)
Time from PET scan to autopsy (months)	3·8 (3·0)	6·6 (6·0)
Participants with Alzheimer's disease	n=20	n=29
Time from onset of symptoms to enrolment (months)	107·4 (46·0)	105·8 (40·9)
Time from diagnosis to enrolment (months)	76·6 (47·4)	77·9 (44·4)
Participants with other dementia	n=11	n=13
Time from onset of symptoms to enrolment (months)	88·5 (58·9)	82·4 (55·9)
Time from diagnosis to enrolment (months)	61·7 (62·7)	54·2 (60·1)
Participants with mild cognitive impairment	n=4	n=5
Time from onset of symptoms to enrolment (months)	70·8 (52·8)	69·2 (45·9)
Time from diagnosis to enrolment (months)	27·3 (31·2)	24·8 (27·6)

Data are number (%) or mean (SD).

Table 1: **Demographic and clinical characteristics of participants**

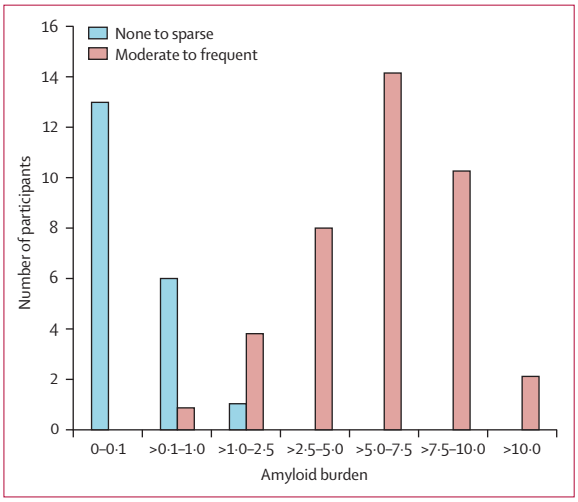

Figure 2: **Frequency distribution of pathological measurements by amyloid burden and by CERAD diagnosis**
Amyloid burden is mean amyloid-β density in the six target cortical regions, as measured with immunohistochemistry at autopsy. CERAD=Consortium to Establish a Registry for Alzheimer's Disease.

PET scan. Two of 110 surviving participants enrolled in the current study withdrew consent and 29 died within 24 months of their original florbetapir PET scan. Family members withdrew consent for autopsy for five of these participants (figure 1). During this period, an additional 24 autopsies became available, and the combined total of 59 participants with a valid florbetapir PET scan and autopsy within 24 months comprised the primary efficacy analysis population. Of these 59 participants, 46 had an autopsy within 12 months (mean 3·8 months [SD 3·0]) of the florbetapir PET scan and comprised the predefined secondary analysis population. The other 13 cases had a mean interval of 16·3 months (2·8) between imaging and autopsy. Common causes of death included cancer, cardiorespiratory failure, chronic obstructive pulmonary disease, heart failure, and end-stage dementia.

Florbetapir was well tolerated and there were no serious adverse events. With the exception of two instances of headache, no adverse event occurred in more than one participant or was judged to be related to treatment.

The mean age of the primary efficacy analysis population was 79·4 years and male and female participants were equally represented (table 1). According to clinical criteria, 12 participants had no cognitive impairment, five had mild cognitive impairment that did not meet the criteria for dementia, 29 had Alzheimer's disease, and 13 had other forms of dementia, including dementia with Lewy bodies, Parkinson's disease dementia, frontotemporal dementia, unspecified dementia, and mixed dementia (table 1). A median of 6·6 months elapsed between the florbetapir PET scan and autopsy. The secondary efficacy analysis population had similar demographic and disease characteristics to the primary cohort (table 1).

Figure 2 summarises the relation between the pathological reference standards as a frequency distribution of the immunohistochemistry results and CERAD classification of neuritic plaque density. Overall, the measurement of amyloid β with immunohistochemistry showed a bimodal distribution with a large measurement range between participants. In participants with a positive CERAD neuropathological finding (moderate or frequent neuritic plaques), the mean, median, or mode amyloid-β density as measured with immunohistochemistry fell between 5·0% and 7·5%, but values for individuals ranged from 1·0% to 14·0%. Similarly, in participants with a negative CERAD neuropathological finding (no or sparse neuritic plaques), the mean, median, and mode amyloid-β density fell between 0 and 0·1%, but values for individuals ranged from 0 to 1·1%.

39 (66%) of 59 participants in the primary efficacy population had moderate or frequent neuritic plaques at autopsy and thus were judged to be positive for amyloid β according to histopathological assessment (table 2). The majority of readers rated the florbetapir PET scans as positive in 36 of these 39 individuals, a sensitivity of 92% (table 3). All 20 participants with no or sparse neuritic plaques at autopsy (negative for amyloid β with histopathological assessment) were assessed as negative by the majority of readers of the florbetapir PET scan and corresponded to a specificity of 100%. The overall accuracy of the majority reading was 95% (table 3). In the 46 participants with an imaging-to-autopsy interval of up to 12 months, the results of the majority reading did not correctly classify one participant, who was positive for amyloid β at autopsy

(table 2). Thus, the sensitivity, specificity, and accuracy were 96%, 100%, and 98%, respectively (table 3). Because specificity was 100% in both clinically normal and cognitively impaired individuals and only one clinically normal participant had moderate to frequent plaques at autopsy, the results were not changed by analysis including only those participants with cognitive impairment (data not shown).

Individual readers also showed both high accuracy and excellent inter-reader agreement. The median and mean sensitivities for the five readers for the 59 cases in the primary efficacy population were 92% and 87%, respectively; the median and mean specificities were 95% (table 3). Notably, 276 (94%) of 295 individual readings agreed with the majority reading (table 3). Fleiss' κ for all inter-reader comparisons was 0·75 (95% CI 0·67–0·83), which showed high inter-reader reliability.

With a prespecified cutoff of at least 1·10 for the semiautomated SUVR, the sensitivity was 97% (38 of 39; 95% CI 85–100), specificity was 100% (20 of 20; 80–100), and accuracy was 98% (58 of 59; 90–100) for detection of moderate to frequent neuritic plaques.

The visual semiquantitative rating of amyloid β by use of florbetapir PET imaging showed a significant positive correlation with post-mortem levels of amyloid β measured with immunohistochemistry in the participants who had an autopsy within 2 years of the PET scan (ρ=0·76; p<0·0001; appendix p 1). A similar relation was present for the subset of participants with less than 12 months between imaging and autopsy (0·79; p<0·0001). No significant difference (p=0·56) was noted between the 35 participants studied by Clark and colleagues[5] (ρ=0·80) and the subsequent 24 individuals (ρ=0·73). Similar to the earlier study, all exploratory measures of correlation were significant; amyloid-β pathology as assessed with florbetapir PET was correlated with pathology at autopsy, irrespective of the methods used to assess PET (semiquantitative visual read or

	Demographics			Florbetapir PET imaging							Post-mortem neuropathology			
	Clinical diagnosis	Age at death (years)	Scan to autopsy interval (months)	Majority reading	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	SUVR	Pathologist's diagnosis*	Immunohistochemistry†	CERAD plaque count‡	Cortical average plaque count§
Negative neuropathological diagnosis (no or possible AD), autopsy <1 year after scan														
1	MCI	48	0	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	1·09	No AD	0·001	0	0
2	AD	82	2	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	0·87	No AD	0·162	0	0
3	NC	62	1	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ+	0·88	No AD	0·008	0	0
4	ODD	73	4	Aβ–	Aβ–	Aβ+	Aβ–	Aβ–	Aβ+	0·91	No AD	0·008	0	0
5	MCI	86	5	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	1·07	No AD	0·008	0	0
6	NC	84	2	Aβ–	Aβ+	Aβ–	Aβ–	Aβ–	Aβ–	0·92	No AD	0·000	0	0
7	ODD	87	1	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	0·81	No AD	0·007	0	0
8	NC	55	3	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	1·09	No AD	0·034	0	1·23
9	NC	93	1	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	1·00	No AD	1·123	0	0·04
10	NC	85	2	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	0·88	No AD	0·008	0	0
11	NC	85	3	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	1·00	No AD	0·002	0	0
12	NC	99	2	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	0·92	No AD	0·029	0	0·33
13	MCI	92	4	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	0·87	No AD	0·011	0	0
14	NC	62	4	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	0·93	No AD	0·005	0	0
15	MCI	66	8	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	1·02	Possible AD	0·720	1	0·58
16	NC	90	3	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	0·99	Possible AD	0·127	3	1·00
17	ODD	104	3	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	0·98	Possible AD	0·521	5	1·71
18	NC	70	5	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	1·00	Possible AD	0·558	5	2·96
Negative neuropathological diagnosis (no or possible AD), autopsy >1 year after scan														
19	NC	93	17	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	0·94	No AD	0·157	0	0·54
20	ODD	62	15	Aβ–	Aβ–	Aβ–	Aβ+	Aβ–	Aβ–	0·72	Possible AD	0·037	1	0·21
Positive neuropathological diagnosis (probable or definite AD), autopsy <1 year after scan														
21	ODD	72	11	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·13	Probable AD	4·890	6	2·42
22	AD	86	3	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·45	Probable AD	3·651	8	1·88
23	AD	92	4	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·64	Probable AD	1·378	13	5·50
24	AD	91	4	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·91	Probable AD	6·143	15	10·92
25	ODD	84	7	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ–	1·84	Probable AD	7·394	17	9·46
26	AD	88	5	Aβ–¶	Aβ–	Aβ+	Aβ–	Aβ–	Aβ+	1·23	Probable AD	4·765	17	15·31
27	ODD	67	6	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·34	Definite AD	7·181	20	10·88

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	Demographics			Florbetapir PET imaging							Post-mortem neuropathology			
	Clinical diagnosis	Age at death (years)	Scan to autopsy interval (months)	Majority reading	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	SUVR	Pathologist's diagnosis*	Immunohistochemistry†	CERAD plaque count‡	Cortical average plaque count§
(Continued from previous page)														
28	ODD	91	10	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ–	1·76	Definite AD	6·078	22	28·21
29	ODD	76	6	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ–	1·16	Definite AD	5·773	23	15·92
30	AD	80	5	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	2·14	Definite AD	9·613	23	21·38
31	AD	79	1	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·38	Definite AD	8·563	23	17·06
32	AD	85	10	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·31	Definite AD	9·867	23	12·42
33	AD	89	6	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·39	Definite AD	1·574	23	12·52
34	NC	82	2	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ–	1·17	Definite AD	9·648	28	21·63
35	AD	86	0	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·56	Definite AD	5·355	28	21·80
36	ODD	77	11	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·11	Definite AD	6·851	30	21·79
37	AD	91	1	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·37	Definite AD	9·372	31	19·50
38	AD	63	9	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·29	Definite AD	8·888	35	26·08
39	AD	89	5	Aβ+	Aβ+	Aβ+	Aβ–	Aβ+	Aβ–	1·27	Definite AD	7·789	36	11·50
40	AD	55	0	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·38	Definite AD	4·651	37	34·63
41	AD	81	3	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ–	1·20	Definite AD	5·338	38	16·15
42	ODD	78	0	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ–	1·17	Definite AD	3·594	42	20·04
43	AD	76	4	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·20	Definite AD	7·395	44	29.04
44	AD	84	5	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·66	Definite AD	8·846	45	29.60
45	AD	69	1	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·63	Definite AD	5·988	46	20·08
46	ODD	88	0	Aβ+	Aβ+	Aβ+	Aβ–	Aβ+	Aβ+	1·21	Definite AD	1·628	56	23·85
47	AD	59	4	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·57	Definite AD	10·376	60	44·46
48	AD	72	0	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·36	Definite AD	5·309	105	70·75
Positive neuropathological diagnosis (probable or definite AD), autopsy >1 year after scan														
49	MCI	61	22	Aβ–¶	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	1·13	Probable AD	1·145	6	6·50
50	AD	89	14	Aβ–¶	Aβ–	Aβ–	Aβ–	Aβ–	Aβ–	1·02‖	Probable AD	0·296	7	2·67
51	ODD	81	18	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ–	1·19	Probable AD	4·375	11	6·96
52	AD	70	20	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·21	Definite AD	14·179	20	12·33
53	AD	85	13	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·24	Definite AD	6·980	20	18·54
54	AD	88	13	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·40	Definite AD	3·740	20	8·58
55	AD	95	18	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·52	Definite AD	6·105	24	20·58
56	AD	103	17	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·13	Definite AD	7·403	24	10·88
57	AD	96	14	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ–	1·14	Definite AD	3·627	27	16·71
58	AD	67	17	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ–	1·41	Definite AD	8·780	35	23·71
59	AD	78	14	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	Aβ+	1·50	Definite AD	8·023	45	25·88

SUVR=standard uptake visual ratio. CERAD=Consortium to Establish a Registry for Alzheimer's Disease. AD=Alzheimer's disease. ODD=other or non-AD dementia. MCI=mild cognitive impairment. NC=cognitively intact normal control. *Derived from the CERAD plaque counts in accordance with CERAD criteria such that a plaque count of 0 in the highest field was assigned the semiquantitative designation of no plaques and a diagnosis of no AD; counts of 1–5 were judged to be sparse and given a diagnosis of possible AD; 6–20 were judged to be moderate, diagnosis probable AD; and greater than 20 were judged to be frequent, diagnosis definite AD. †Mean amyloid-β density: mean percentage of grey matter in the six target cortical regions containing aggregated amyloid-β as detected by use of 4G8 immunohistochemistry at autopsy. ‡Plaque count in highest field from CERAD diagnostic regions. §Average plaque count in highest field from six cortical regions (frontal, temporal, parietal, precuneus, anterior cingulate, and posterior cingulate) was used in imaging assessment for calculation of SUVR. ¶Majority binary reads of PET scans discordant with the pathologist's diagnosis. ||SUVR discordant with the pathologist's diagnosis.

***Table 2*: Demographic, imaging, and neuropathological data for participants**

	Participants with <12 months from scan to autopsy (n=46)			All autopsy participants (n=59)		
	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy
Majority reading	27/28; 96% (80–100)	18/18; 100% (78–100)	45/46; 98% (87–100)	36/39; 92% (78–98)	20/20; 100% (80–100)	56/59; 95% (85–99)
Median reader (range)	96% (75–100)	94% (89–100)	96% (80–98)	92% (69–95)	95% (90–100)	93% (76–95)
Mean of readers (SD)	91% (10·0)	96% (4·6)	93% (7·3)	87% (10·4)	95% (3·5)	90% (7·9)

Data are n/N; % (95% CI), unless otherwise indicated.

***Table 3*: Diagnostic agreement between visual binary florbetapir PET measurement by five readers and neuropathologist's rating of neuritic amyloid plaques**

SUVR) or autopsy material (immunohistochemistry or silver staining; data not shown). Although no adjustments for multiplicity were planned for the analyses of correlation, significance in all tests was retained after post-hoc Bonferroni adjustments, dividing the α equally between the four correlation analyses (data not shown).

Discussion

The results of this study, designed as an extension of our previous study,[5] reaffirmed the relation between florbetapir PET and brain amyloid pathology assessed at autopsy. The results also showed agreement between the binary qualitative florbetapir PET scan visual rating (amyloid positive or amyloid negative) and the neuropathological classification (moderate or frequent *vs* no or sparse neuritic plaques) at autopsy in participants with a range of amyloid-β pathology that is similar to the range reported in a clinical population of patients with cognitive impairments (panel).[16,17]

The correlation between the semiquantitative visual assessment of florbetapir PET scans and mean density of amyloid β on immunohistochemistry was similar to that previously reported[5] and there was no significant difference between the correlations seen in the original 35 participants and the 24 additional individuals in this study. Similar to the results of our previous study, a significant positive relation was noted between amyloid burden on PET and at autopsy, irrespective of the method used to assess florbetapir PET images (semiquantitative visual assessment or SUVR) or amyloid density at autopsy (immunohistochemistry or neuritic plaque score with silver staining). The similarity to the previous results was not unexpected because of the overlap of 35 cases between the two studies. However, the finding that the associations were not significantly different among the 35 original and the 24 new cases confirms the reliability of the initial finding.

Although estimates of the correlation between PET imaging scores and histopathological assessment of amyloid burden was 0·7–0·8, this accounts for only 50–60% of the mutual variance. This was not unexpected because the antibody used for the immunohistochemistry binds to a specific domain of amyloid β, and presumably to all amyloid present, unlike florbetapir, which is specific for fibrillar amyloid. Additionally, immunohistochemistry is an in-vitro technique in which the ligand presumably has access to the tissue without clearance or metabolic interaction, which is not the case with florbetapir imaging.

A limitation of the previous study[5] was the use of a semiquantitative categorical (0–4) rating of the florbetapir PET scan. By contrast, the binary interpretation in the present study (including calculations of sensitivity and specificity with respect to neuropathological findings) corresponds to the interpretation of interest in a clinical setting. In the present study, majority-score binary visual ratings were 92% sensitive and 100% specific for distinguishing cases with moderate to frequent plaques from those with no or sparse plaques. Moreover, individual readers assigned scores in the same range as the majority score with a high inter-rater agreement, showing the accuracy and reliability of the individual readers. However, the results, obtained with only five imaging readers, might not be predictive of the scoring of all nuclear medicine physicians. Although the training methods were similar to those recommended for community use and the readers had a range of experience, additional studies are needed to monitor success in community practice.

Because Alzheimer's disease is a progressive neurological disorder, the relation between post-mortem pathological changes and actual changes in the brain at the time of a PET scan might decrease with increasing scan-to-autopsy intervals. For this reason the original protocol[5] and a prespecified analysis in the current protocol focused on participants who had autopsy within 1 year of the florbetapir PET scan. Sensitivity in the present study was 96% for cases who had autopsy within 1 year and 92% within 2 years. The two individuals (cases 49 and 50) who accounted for this difference had borderline-positive neuritic plaque counts (seven and six plaques per high-power field; table 2). A limitation of PET amyloid imaging is that only the present density of amyloid β in the brain is detected and individuals at risk of becoming amyloid-β positive in the future cannot be identified. Florbetapir scans nearer to death might have been positive in cases 49 and 50. In previous studies of ^{11}C-PIB, the amyloid-β-negative status changed to positive at a rate of about 3% per year.[18]

In the current study, we also assessed the relation between SUVR and neuritic plaque density. Based on values from a series of young participants who were cognitively normal, Joshi and colleagues[11] have proposed a cutoff value of 1·10 to distinguish normal from abnormal scans. In our study, all the cases with no or sparse plaques at autopsy had SUVR values of less than 1·10, and all but one with moderate or frequent plaques at autopsy had SUVR values greater than 1·10.

The results of this study are the first to provide direct evidence of the sensitivity and specificity of PET imaging by comparison with neuropathological findings. Brain amyloid-β plaques have been assessed by use of histopathology in individuals imaged with ^{11}C-PIB or flutemetamol in other studies,[6–10] but none were designed to establish a threshold relation between the PET imaging results and a criterion level of amyloid pathology. Moreover, these previous studies had either biopsy samples from single cortical regions from living patients or longer intervals from imaging to autopsy and the studies, with three to ten individuals, were much smaller than this study. Nevertheless, the results of these smaller studies have shown an association between tracer uptake and amyloid pathology, consistent with our current and previous findings.[5]

Panel: **Research in context**

Systematic review

We searched PubMed with the terms "amyloid imaging" AND "autopsy". The search was repeated with the terms "Pittsburgh compound B", "florbetapir", "florbetaben", and "flutemetamol" replacing "amyloid imaging". As of March 30, 2012, amyloid imaging and histopathology results for more than one case had been reported in only six studies.[5–10] The largest of these[5] included 35 of 59 participants in the current study and the results showed a relation between amyloid as assessed with florbetapir PET imaging and post-mortem amyloid burden assessed with immunohistochemistry. The remaining studies all had ten or fewer cases each. Thus, our study is the first of sufficient size, with a-priori specified criteria that allow measurement of the sensitivity and specificity of amyloid imaging for detection of the accumulation of neuritic amyloid plaques.

Interpretation

In this cohort of patients nearing the end of their lives, florbetapir PET scans were 92% sensitive and 100% specific, with an inter-rater reliability (κ) of 0·76. The results suggest that binary visual interpretation of florbetapir PET could be used to reliably distinguish patients with no or sparse plaques (amyloid-β negative: inconsistent with a diagnosis of Alzheimer's disease) from patients with moderate to frequent plaques (amyloid-β positive: consistent with the presence of Alzheimer's disease). Additional research is needed to understand the prognostic implications of moderate to frequent plaque density. As with any diagnostic imaging test, physicians should use the test only for the intended purpose, and should carefully evaluate and interpret the results in the context of other relevant diagnostic information.

One potential limitation of this study is that it was undertaken in participants nearing the end of their lives, who were generally older and in poorer health than is the typical individual seeking diagnosis of cognitive impairment in a community setting. However, the density of amyloid β seen by use of immunohistochemistry in this study population was likely to have included the range of amyloid densities occurring in patients seeking diagnosis. According to the CERAD plaque classification, 15 individuals (25%) had no neuritic plaques, five (8%) had sparse plaques, nine (15%) had moderate plaques, and 30 (51%) had frequent neuritic plaques (table 2). This range of amyloid pathology is similar to that reported for a community-based population with mild cognitive impairment or Alzheimer's disease.[16,17] Thus, although impossible to know the amyloid levels in an unbiased sample of the entire elderly population (who have a wide range of life expectancies), we believe the participants studied here are representative of the range of pathology likely to be seen in individuals referred for diagnostic imaging, and, in view of the potential complications resulting from continued accumulation of amyloid pathology over time, we believe the enrolment strategy in this study is appropriate for assessment of the relation between PET imaging and post-mortem amyloid pathology.

Another potential limitation of this study is that both the amyloid imaging and histopathology results were distributed bimodally so there were few cases with borderline amyloid pathology, whether assessed with florbetapir PET scan, immunohistochemistry, or plaque counts. Bimodal distributions of PET imaging signals have previously been noted in participants in our own studies[19] and in other patient cohorts.[20,21] Results of earlier studies suggested that patients might transition rapidly from amyloid negative to amyloid positive,[18] which would account for the few individuals with borderline amyloid PET signal in cross-sectional studies.[19–21] Moreover, our theoretical understanding of amyloid β and experimental data suggest this transition might happen several years before individuals have clinical symptoms of cognitive impairment (ie, participants who are clinically normal would not usually be referred for diagnostic imaging). Additional studies will be needed to assess how frequently borderline scans are obtained in clinical practice, and the timeframe for progression from negative to borderline positive.

The bimodal distribution of pathology raises the question of whether a lower sensitivity might have been obtained if more participants had intermediate pathology (ie, sparse to moderate plaques). The two cases with amyloid-negative PET scans and borderline positive neuritic plaque density might indicate the lower limit of scan sensitivity, or the continued accumulation of amyloid over the 1–2 years of follow-up. Among patients coming to autopsy within 1 year of PET scan, the classification (amyloid-β positive or negative) was consistent with the autopsy results in nine of ten cases with sparse or moderate plaques. The discrepant case (number 26) was a patient with severe dementia (mini-mental state examination score 9) who had the highest amyloid burden of all the cases with moderate neuritic plaques at autopsy, suggesting the difficulty might be due to other scan features (eg, atrophy) rather than a borderline amyloid burden.

The clinical significance of amyloid burden as measured with florbetapir PET must be interpreted in the context of other relevant diagnostic information. In the current study, even the majority reading score was not perfect for prediction of which individuals would be positive for amyloid at autopsy and one individual reader showed notably lower sensitivity than did the other readers. Images might be difficult to read, and errors, especially false negatives, will arise because of borderline amyloid levels, image noise, brain atrophy, or image blur (issues that might have been exacerbated in this end-of-life population). Thus, as with any diagnostic imaging test, physicians should use the test only for the intended purpose, and should carefully assess and interpret the results in the context of other relevant diagnostic information.

PET imaging of amyloid alone does not establish a diagnosis of Alzheimer's disease or other neurological disorders. However, taken together with other clinical information and results of diagnostic tests, florbetapir PET might be an aid to diagnosis. A negative florbetapir PET scan is inconsistent with a contemporaneous neuropathological diagnosis of Alzheimer's disease, thus reducing the likelihood that a patient's cognitive impairment is due to this disorder. A positive florbetapir PET scan indicates the presence of amyloid in a density noted in patients with Alzheimer's disease, but this density can arise in other neurological disorders or in elderly individuals who are cognitively normal.[8,9,16] Additional research is needed to understand the significance and prognostic implications of moderate to frequent plaque densities in people who are clinically normal and those with other comorbid disorders such as dementia with Lewy bodies or Parkinson's disease.

In conclusion, the results of this study confirm the results of Clark and colleagues.[5] The correlation between florbetapir PET imaging and post-mortem amyloid burden supports the conclusion that florbetapir PET might be useful for imaging of amyloid-β neuritic plaques in the brains of patients with cognitive impairment. The sensitivity, specificity, and inter-rater reliability among the five nuclear medicine physicians suggest florbetapir might distinguish patients with no or sparse plaques from those with moderate to frequent plaques.

Contributors

CMC, MJP, TGB, BJB, JAS, AA, APC, MJK, MAM, and DMS were involved in study conception and design. CMC, MJP, TGB, BJB, EMR, JAS, and MAM supervised the study. TGB, BJB, REC, PMD, ASF, EMR, MNS, JAS, CHS, AA, AJD, MLF, and MAM were involved in data acquisition. CMC, MJP, TGB, BJB, JAS, MJK, MAM, and DMS did the data analysis and interpretation. ML did the statistical analysis. CMC and MJP drafted the report. ADJ and ML prepared the figures and tables. PMD, REC, and DMS obtained funding for the study. MJP, TGB, BJB, REC, PMD, ASF, EMR, MNS, CHS, JAS, AA, APC, MLF, ADJ, MJK, ML, MAM, and DMS were involved in the critical revision of the report for intellectual content.

Conflicts of interest

CMC, MJP, AA, APC, MLF, ADJ, MJK, ML, MAM, and DMS are or were employees of Avid, a division of Eli Lilly, and formerly held Avid stock or options. TGB has received funding related to the topic of this report from the National Institute on Aging (grant P30 AG19610), Arizona Department of Health Services (contract 211002 awarded to the Arizona Alzheimer's Research Center), Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901 and 1001), Avid Radiopharmaceuticals, Bayer Healthcare, and GE Healthcare. BJB has received compensation and shares from Biospective. REC reports membership of the medical advisory board for GE Healthcare from 2003 to 2008; being a consultant for GE Healthcare from 2003 to 2008; receiving a research grant from GE Healthcare in 2010; receiving funding for a clinical trial from Molecular Insights Pharmaceuticals in 2010; serving on a medical advisory board for Molecular Insights Pharmaceuticals from 2004 to 2009; serving on a medical advisory board and receiving a grant from Avid to support his participation in this study; serving on a medical advisory board and as a consultant to Eli Lilly; and serving on a medical advisory board for Bayer. PMD reports receiving research grants related to this project (awarded to Duke University); currently or previously serving as an adviser to Forest, Bristol-Myers Squibb, Avid Radiopharmaceuticals, Lundbeck, Medivation, Pfizer, Elan, Eli Lilly, Bayer, Neuroptix, Neuronetrix, Sonexa, Accera, TauRx, Myriad, National Institute on Aging, AstraZeneca, Labopharm, Clarimedix, National Institute of Mental Health, National Institute of Neurological Disorders and Stroke, Alzheimer's Association, Alzheimer's Foundation, Rutgers University, and the University of California; owning stock in Sonexa and Clarimedix; and receiving a grant from Avid (awarded to Duke University) for his participation in this study. ASF has served as a consultant to Lilly and Avid, and received grant funding from Avid. EMR reports serving as a scientific adviser to Sygnis, AstraZeneca, Bayer, Eisai, Elan, Eli Lilly, GlaxoSmithKline, Intellect, Link Medicine, Novartis, Siemens, and Takeda. He has had research contracts with AstraZeneca and Avid/Eli Lilly; a patent pending for a biomarker strategy to evaluate preclinical treatments for Alzheimer's disease (through Banner Health); and research grants from the National Institute on Aging, an anonymous foundation, Nomis Foundation, Banner Alzheimer's Foundation, and the State of Arizona. MNS reports serving in a consulting or advisory capacity for Eli Lilly, Amerisciences, Takeda, Eisai, Pfizer, GlaxoSmithKline; receiving royalties from Wiley, FT Pearson Press, and Amerisciences; and receiving contracts and grants from Celgene, Ceregene, Bayer, Baxter, Bristol-Myers Squibb, GE Healthcare, Eli Lilly, Pfizer, Wyeth, Janssen, Elan, Avid, Genentech, Medivation, and Eisai. CHS reports serving on speaker bureaus for Novartis, Forest, and Accera, and as a consultant to Novartis and Eli Lilly. JAS reports being a consultant for Avid Radiopharmaceuticals and receiving compensation for services.

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