Amyloid Imaging of Alzheimer's Disease Using Pittsburgh Compound B

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The advent of human amyloid imaging represents a research breakthrough in Alzheimer's disease (AD). It is now possible to detect the early stages of cerebral amyloidosis, a major pathologic component of AD, in living humans using positron emission tomography (PET). This technology will likely enable earlier AD diagnosis, but further research is required to determine whether a positive amyloid PET scan predicts imminent decline in questionably or mildly impaired individuals, and whether amyloid PET can be used to track the efficacy of emerging antiamyloid therapeutic agents. Initial human data are encouraging but suggest that individual amyloid PET findings should be interpreted cautiously, because cerebral amyloidosis precedes and does not equate with either clinical AD or pathologic criteria that define AD.

Introduction

Alzheimer's disease (AD) affects one of every 10 individuals in the United States over the age of 65 years and poses a major threat to public health in an aging population with increasing life expectancy. The pathophysiology of AD has been vigorously investigated over the past decade. Critical insights have been gained into the neurochemistry and biology underlying the two hallmark AD pathologic lesions, which are aggregates of proteinaceous fibrils, known as amyloid-β (Aβ) plaques and neurofibrillary tangles [1].

In the case of amyloid plaques, encouraging findings from transgenic AD mouse models led the way to specifically targeted therapeutic approaches that are currently in clinical trials [2]. There is unprecedented optimism, but unfortunately clinicians and scientists have been unable to identify and track the hallmark pathologies of AD in living humans. This is a significant barrier to exploration of basic questions relating pathophysiology and symptomatology. For example, a rational account of how amyloid deposits relate to the cognitive and behavioral syndrome of AD is lacking, and a central, persistent enigma in AD investigation is the poor correlation between brain amyloid burden assessed at autopsy and the severity of clinical impairment. Does the accumulation of certain forms of amyloid precede or follow the onset of clinical impairment, and do the amounts and anatomic distribution relate to the cardinal symptoms of memory and executive impairment? Can we expect that the removal of these deposits will produce clinical improvement? How does amyloid imaging meet or fall short of the ideal characteristics of a validated biomarker for drug development? Can amyloid imaging serve as a surrogate endpoint for therapeutic trials? Answers to these questions will aid the development of treatments that target the most critical stages of pathologic progression in AD.

Over the past two decades, efforts to develop a human molecular imaging method for AD pathology have produced numerous compounds that have reached various stages of maturity [3••,4,5]. Many of these compounds have the potential to serve as useful biomarkers of disease presence and progression, but to date only one, the thioflavin derivative Pittsburgh Compound B (PIB), has entered clinical imaging research at multiple research sites involving several hundred patients [6]. We review the background and recently reported data on the use of PIB, specifically 1) the basis for expecting amyloid imaging to be a valuable tool in AD, 2) preclinical animal and postmortem evidence supporting the use of PIB for amyloid imaging, and 3) human studies to date in which ^{11}C PIB positron emission tomography (PET) has been examined in relation to clinical classification, 18 fluoro-2-deoxy-D-glucose (FDG) metabolism, cerebrospinal fluid (CSF) findings, and structural imaging.

Cerebral Amyloid and AD Pathology

There is compelling evidence that a key pathologic process in AD occurs when excessive Aβ protein accumulates, due either to excessive production or to insufficient clearance from the brain. All of the known autosomal dominant genetic mutations that result in clinical AD are known to increase production of Aβ [7], and Aβ deposition in plaques occurs invariably in the brains of patients with

clinical AD [8]. Amyloid accumulation and plaque deposition is a complex process involving successive stages of polymerization and aggregation. Amyloid imaging with PIB, as well as other candidate compounds, is specifically directed at particular forms of amyloid, which should be understood in the context of a progression from the soluble Aβ peptide through several intermediate stages to the dense core neuritic plaque. Unfortunately, a consistent neuropathologic terminology for the various stages of plaque morphology has not gained wide acceptance, and the natural history of brain amyloid formation is still a topic of active investigation.

Aβ is the major component of the particular amyloid deposited in AD plaques and is formed in the brain when a large transmembrane protein (amyloid precursor protein) is snipped at a particular site along the peptide chain [9]. The resulting Aβ peptide, either the 40 or the more amyloidogentic 42 amino acid species, can self-assemble into oligomeric forms, which can be trimeric, tetrameric, and so forth, and which can diffuse throughout the brain. Soluble forms of Aβ undergo β-sheet conformational change, forming intermediates such as spheroids and "protofibrils," but eventually form fibrillar aggregates. β-Sheet protein folding is responsible for the characteristic, traditional staining properties of amyloids (eg, the apple-green birefringence with Congo red). The β-sheet is also responsible for the binding of thioflavin-related compounds such as PIB. Recent studies indicate that there are at least three separate binding sites for PIB on the Aβ polymer fibrils, each with its own affinity [5], suggesting a possible direction for refinement of amyloid imaging compounds in the future. Although the β-sheet conformation is the structure labeled by thioflavins and by PIB, it is postulated that Aβ monomers are in steady-state equilibrium with fibrillar forms. This may mean that imaging markers of fibrillar amyloid such as PIB are reasonable indicators of the presence and concentration of soluble or prefibrillar forms as well; however, this relationship remains to be confirmed.

Numerous mechanisms of Aβ neurotoxicity have been proposed, implicating both fibrillar and soluble forms. Fibrillar forms of Aβ can directly disrupt neuropil, resulting in markedly altered axonal trajectories [10,11]. Amyloid plaques have been shown to impair evoked synaptic responses, causing potential asynchrony of convergent inputs [12], and decreasing Aβ can acutely restore long-term potentiation and improve behavioral deficits in animals [13–16]. Recent reports, however, have implicated soluble forms of Aβ in neurotoxicity. For example, a 56-kD form of oligomeric Aβ that was raised in transgenic mouse models of AD was reported to produce memory impairment when administered to naïve rats [17]. Whether the upstream, soluble forms of Aβ are both toxic and responsible for the human disease is not yet established, and more than one form of Aβ could well be involved. Some have suggested that fibrilization could act to sequester toxic soluble species, such that amyloid fibrils represent a primarily protective form. These issues are under active investigation and the outcome may have implications for the development of optimal imaging agents. Although much of the pathogenesis of AD remains a mystery, the potential value of amyloid imaging was emphasized in preliminary reports that immunotherapeutic approaches to remove amyloid may have positive effects on memory measures in AD patients [18,19].

The precise interpretation of PIB binding in PET images can be informed by the extensive neuropathologic literature in AD and prodromal AD. Plaque amyloid is typically demonstrated in postmortem brains with standard histologic methods such as the Bielschowsky silver stain and thioflavin S, and with immunocytochemical techniques used for staining particular components of plaques. These methods have been used to characterize plaque morphology and evolution. More primitive insoluble forms of amyloid take a variety of forms that include the "diffuse" amyloid plaque. This form is commonly seen at postmortem examination in many normal individuals above a certain age range as well as in patients who were identified as demented during life [20,21,22••,23,24,25••]. Diffuse plaques have finely fibrillar elements patients but lack dense cores and neuritic elements. Many investigators suspect that diffuse plaques in nondemented elderly are an antecedent form of the same pathologic process that results in AD. Initial correlations of PIB PET with postmortem findings are consistent with the notion of PIB binding as a biomarker for early fibrillar forms of amyloid plaque. Whether clinical impairment can be predicted to occur within a particular time-frame after the first appearance of PIB-positive PET images is a timely opportunity for clinical neuroimaging research.

More mature and complex plaque morphologies contain abundant β-sheet amyloid and thus would be expected to bind PIB. Plaques are frequently associated with dystrophic, tau-positive neurites and often with surrounding reactive astrocytes and microglia, as well as evidence of an inflammatory reaction. They frequently contain a dense central core of amyloid, which itself may be somewhat less available for binding PIB compared to other, less tightly compacted portions of the plaque. Another form of amyloid pathology very commonly seen in AD postmortem tissue is angiopathy, a lesion of small- to medium-sized vessels in which β-sheet amyloid appears in parenchymal and leptomeningeal vessel walls and binds PIB [24].

Although Aβ plaques are clearly a pathologic hallmark of AD, a single histopathologic standard for determining "definite AD" has not been agreed upon, and complex, parallel lesion grading schemes have been developed for both the amyloid plaque and the neurofibrillary tangle (The National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's, 1997). The portions of this grading system that relate

to amyloid pathology, the "Consortium to Establish A Registry for Alzheimer's Disease" (CERAD) criteria, specify that neuritic plaques, and not diffuse plaque amyloid, are required to fulfill the criteria. Therefore, PIB binding of plaque amyloid would likely be seen in many individuals who would not meet CERAD criteria. In other words, it may be best to regard PIB PET images as reflecting "cerebral amyloidosis" rather than reflecting histopathologic criteria that are based on more advanced forms of plaque morphology [25••,26]. This poses a critical challenge for clinical neuroimaging research, to relate PIB positivity to both clinical and pathologic outcomes and determine the predictive value of PIB as a putative biomarker of antecedent AD.

In summary, β-amyloid is a key component of AD pathology, and thioflavin derivatives such as PIB bind β-sheets and, therefore, attach to an early form of the amyloid plaque that is a hallmark of the disease.

Amyloid Imaging Compounds—PIB: Chemistry and Animal Studies

A number of molecular imaging agents for the in vivo detection of AD pathology are under active development for use with PET or single photon emission computed tomography (SPECT). Promising agents include the aminonapthalene derivative FDDNP [27–29]; the Congo Red styrylbenzene derivatives BSB [30], X34 [31], and stilbene [32]; and thioflavin-T derivatives IMPY [33] and PIB [3••].

PIB specifically binds to β-pleated sheets in individual amyloid plaques in mouse models of AD [34] as well as in humans [25••]. It crosses the blood-brain barrier rapidly and in sufficient amounts after intravenous administration, and has a high binding affinity for aggregated Aβ. PIB is rapidly metabolized in plasma to highly polar compounds that are not likely to enter the brain. In mouse studies, 95% of the radioactivity in the brain is recovered as the parent compound [3••]. Postmortem tissue studies have indicated that PIB binds specifically to amyloid-laden portions of an AD brain, and that binding was displaceable (ie, blocked by an excess of a substance that is a chemical analogue of PIB). In addition, binding was at a very low, nonspecific level in cortex of a cognitively normal elderly control brain [35]. Human use of PIB in trace concentrations was preceded by a toxicity assessment using a PET-microdosing protocol [36]. Toxicologic studies included genotoxicity (chromosomal aberration, mouse lymphoma mutagenesis, bacterial reverse mutation assay, and mouse micronucleus assay), single dose toxicity in rats, and cardiopulmonary physiology in the rhesus monkey. No toxic effects of PIB were observed [36]. It is estimated that approximately 500 humans have now received PIB without any significant adverse events [6].

PIB PET: Human Studies

The form of PIB currently in use is radiolabeled for PET with ^{11}C. This material requires a cyclotron on site due to the short half-life (20.4 minutes) of ^{11}C. Future developments may result in the availability of a form of PIB that is labeled with the longer half-life of ^{18}F (110 minutes), which would theoretically permit more widespread availability. It is also possible that a single photon emitting agent such as ^{123}I-IMPY will be available at some time in the future and that amyloid imaging would be possible using the more widely available SPECT cameras [33,37].

As PIB is currently implemented in most research settings, subjects are positioned in the scanner, injected with 10 to 15 mCi of ^{11}C PIB, and then scanned using a dynamic acquisition that lasts typically between 60 and 90 minutes. Depending on the particular research protocol, subjects may or may not have arterial catheterization. If a semiquantitative dynamic method such as the Logan graphical analysis technique is used for PET pharmacokinetic data analysis [38], arterial catheterization can be avoided. The Logan method uses a reference tissue instead of an arterial input function and has been validated for PIB [39]. The reference tissue used in several studies to represent nonspecific uptake is the cerebellum, a choice that is justified on the basis of the lack of fibrillar amyloid in the cerebellum [40,41]. An important advantage of the tissue reference–based method is that arterial catheterization is not needed. When this approach is taken, both subject burden and technical difficulty are reduced, and resulting data are useful and comparable to the arterial input–based methods [42].

The first report of PIB PET in humans appeared in 2004 and compared AD patients with normal older controls [25••]. Subsequent reports have found remarkably similar results in comparing AD patients with normal controls [25••,39,42,43••,44]. PIB enters the brain rapidly and, in most normal subjects, is cleared rapidly from all areas except hemispheric white matter and portions of thalamus and brain stem (Fig. 1). In AD patients, PIB is retained in association neocortex at levels 1.5- to twofold higher than white matter. The distribution of PIB specific binding (ie, retention) in AD has substantial regional specificity, such that binding in certain brain regions (eg, medial temporal lobe and primary sensori-motor cortex) is typically at lower levels, whereas other regions (eg, association parietal cortices) have very high levels of binding. The set of regions that are most PIB positive are the association isocortical areas of the medial ("precuneus") and lateral parietal (Brodmann 7), lateral temporal (Brodmann 37/21), frontal (Brodmann 9/10) extending into gyri recti (Brodmann 11), and the allocortical anterior and posterior cingulate (Brodmann 23/24/31/32) [25••,39,42,43••,45]. These are areas known to harbor significant levels of amyloid plaque on postmortem examination.

More recently, elevated PIB binding has been reported in abstract form in autosomal dominant familial AD (FAD) due to the Presenilin-1 (PS-1) mutation [45,46].

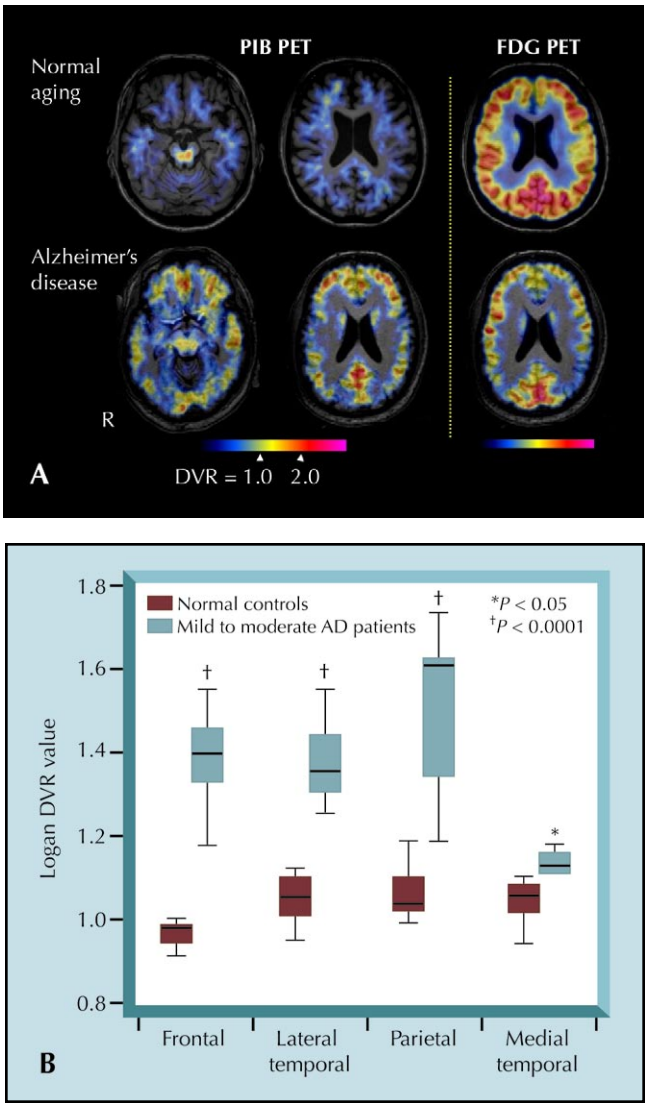

Figure 1. A, Images of the distribution volume ratio (DVR) of Pittsburgh Compound B (PIB) using positron emission tomography (PET) from two transaxial planes superimposed on T1-weighted MRI (at left and center), and 18 fluoro-2-deoxy-D-glucose (FDG) PET standardized uptake value (SUV) image (at right) superimposed on T1-weighted MRI from a single plane, co-registered with adjacent PIB PET image. The *top row* shows images from a normal older control subject showing no PIB retention in cortical regions, low levels of binding in white matter, and normal FDG metabolism. The *bottom row*, in contrast, shows images from an Alzheimer's disease (AD) patient and reveals abundant PIB binding in associated neocortical regions, including frontal, parietal, and precuneus. FDG PET demonstrates parietal hypometabolism. **B,** Box plot of Logan DVR values determined in regions-of-interest (ROI) in a group of older normal controls (NC; $n = 17$) and mild to moderate AD patients ($n = 9$). Greater PIB binding in AD was seen in frontal, lateral temporal, and parietal regions compared with NC ($P < 0.0001$). Medial temporal lobe binding was modestly elevated in AD ($P < 0.05$). The central box shows the date between the upper and lower quartiles, with the median represented by the line. The height of the line is the interquartile range (IQR); the "whiskers" extend from the upper end and lower quartiles to a distance of 1.5 IQR away or to the most extreme data point within that range, whichever is closer.

In these studies, PIB binding was elevated in FAD and sporadic AD patients in a similar distribution within association neocortical regions. Of particular interest, asymptomatic PS-1 mutation carriers also had elevated cortical PIB binding but at levels intermediate between symptomatic AD and normal older controls, indicating that cortical amyloid deposition could be detected during the prodromal phase of AD [45,46].

PIB Imaging in Mild Cognitive Impairment

Other studies of amyloid deposition in prodromal AD have focused on mild cognitive impairment (MCI), a heterogeneous condition in which approximately 15% of subjects convert to AD each year [47]. Initial reports indicate that the amount of PIB binding in MCI subjects is bi-modally distributed, with one subset of subjects showing abundant neocortical PIB binding (PIB positive) and the other showing only low, nonspecific binding (PIB negative), which indicates lack of fibrillar amyloid deposition [42,48,49]. These initial reports indicate that 50% to 60% of MCI subjects are PIB positive [6,42,48,49]; however, the total number of such subjects reported to date remains small. In one such study, Nordberg et al. [49] reported that 62% of 21 amnestic MCI subjects were PIB positive, and that five of the PIB-positive subjects had converted to a clinical diagnosis of AD during the initial follow-up period. These data raise the possibility that amyloid imaging could identify MCI subjects who are more likely to progress over a specified time to AD.

The significance and fate of the PIB-negative MCI subjects is a critically important issue, because PIB imaging may cast doubt on the specific pathologic basis for their impairment. There are at least two distinct explanations for PIB negativity in MCI subjects. First, it is possible that for a fraction of MCI subjects the cause of the impairment is a non-AD process, such as cerebrovascular pathology, medial temporal sclerosis, tauopathy, or other process. Recent postmortem studies

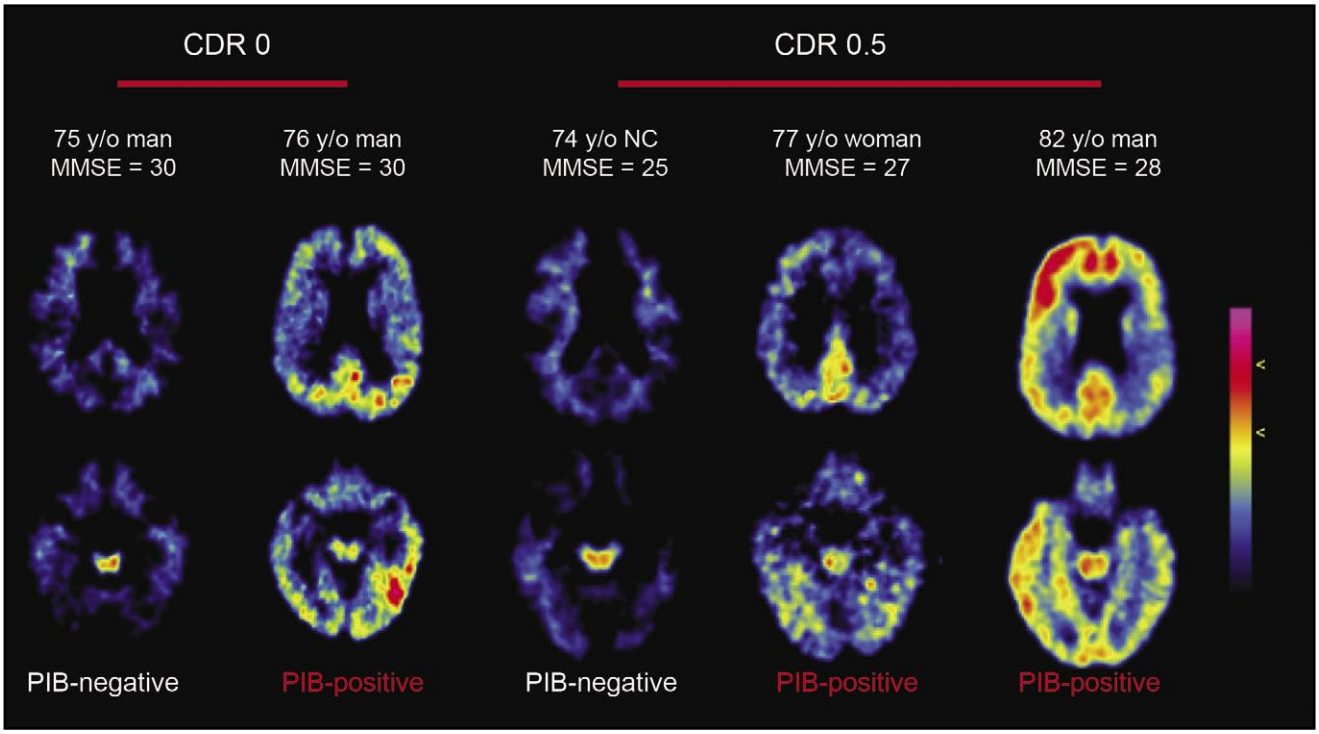

Figure 2. Pittsburgh Compound B (PIB) positron emission tomography (PET) images from two transaxial levels representing normal control (NC) subjects (Clinical Dementia Rating [CDR] score of 0) and mild cognitive impairment subjects (CDR score of 0.5). These images exemplify the observation that specific PIB binding, indicating the presence of fibrillar amyloid-β, has been observed in subsets of both normal control and mild cognitive impairment subjects. In addition, the PIB-positive examples demonstrate the individual variability (eg, asymmetry) seen in anatomic distribution of PIB binding. MMSE—Mini-Mental Status Examination.

support this possibility and have called attention to the heterogeneity observed in the pathology seen in subjects with MCI. Among individuals who fulfilled criteria for MCI near the time of death, seven of 15 had minimal amyloid (Grade 0/3 diffuse plaques) [50]. In a parallel study of MCI patients who progressed to overt dementia, 29% had a non-AD primary pathology [51]. An alternative explanation is that some of the PIB-negative MCI subjects may be in earlier stages of the AD process, when prefibrillar amyloid (oligomeric soluble Aβ or nonfibrillar diffuse insoluble plaque) that is not detected by PIB might be predominant. Although this seems less likely, there is some biologic evidence that amyloid can be rapidly deposited [52], and the cross-sectional MCI data to date are not inconsistent with a steep rise in PIB binding over the course of MCI. Serial PIB imaging with detailed clinical follow-up will be required to help resolve these issues. Ultimately, postmortem examination of subjects who were imaged during life will be required to determine whether image findings represent antecedent AD.

PIB PET in Normal Older Subjects

Initial studies have demonstrated cortical PIB binding in approximately 15% to 20% of individuals who are apparently cognitively normal [42,43••,53]. This finding is not surprising because amyloid plaques are commonly seen in the brains of a subset of older nondemented individuals at autopsy [21,54,55], and it is likely that most of these individuals would be PIB positive. Whether PIB positivity is a meaningful harbinger of impairment and/or dementia in these subjects will require continued observation, but there are several lines of evidence supporting this possibility. One is a recent report by Bennett et al. [55] of a significant relationship between the presence of AD pathology and subtle deficits in episodic memory among clinically normal older individuals [55]. In addition, it is possible that PIB-positive older subjects are very mildly impaired but are able to tolerate a certain amount of amyloid because of cognitive reserve (ie, certain individuals may be able to maintain memory performance in the face of amyloid deposition that would be disabling in others). An initial series of normal control subjects (Clinical Dementia Rating [CDR] score of 0) whose memory test scores were adjusted according to premorbid IQ revealed an excess of PIB-positive subjects in the subset of normals who were very mildly impaired (Fig. 2) [48]. This suggests that PIB PET may detect amyloid loads in individuals with high cognitive reserve who are able to tolerate the amyloid and score in the normal range on memory tests when conventional test norms are applied. Whether such findings represent antecedent AD in normal subjects remains to be determined.

PIB PET in Relation to Cognitive Performance and Compared with Other Putative Biomarkers of AD: CSF, MRI, and FDG Studies

An important unresolved issue in AD neuropathology is that despite the importance of amyloid in the disease process, neither total amyloid burden nor plaque location, assessed at postmortem, is well correlated with clinical impairment. For the first time, this correlation can be investigated in vivo using PIB PET. An initial series investigated the correlation of cortical PIB binding with cognitive testing across all PIB positive subjects (ie, normal, MCI, and AD patients) [48]. In this study, PIB binding was strongly associated with worse performance on the Mini-Mental Status Examination (MMSE) and on the Free and Cued Selective Reminding Test memory scores (FCSRT). In addition, there was a significant correlation between neocortical PIB binding and FCSRT among only the nondemented subjects, suggesting that in mildly impaired or unimpaired individuals there is a correlation between amyloid burden and a sensitive measure of memory impairment. These findings will require confirmation but suggest that amyloid load data in mildly impaired or unimpaired subjects may differ from autopsy-derived data, which are more relevant to later stages of disease.

To date, there have been few comparisons of PIB PET imaging with other putative biomarkers of AD. Fagan et al. [43••] compared CSF $A\beta_{1-42}$ and other CSF measures to PIB binding in a group of subjects that included cognitively normal (CDR of 0, n = 18), very mild (CDR of 0.5, n = 3), mild (CDR of 1, n = 2), or moderate (CDR of 2, n = 1) dementia [43••]. Subjects fell into two non-overlapping groups, regardless of clinical diagnosis: those who were PIB positive had the lowest CSF Aβ levels, consistent with the "sink" hypothesis that plaque amyloid sequesters Aβ [43], whereas PIB-negative subjects had the highest levels of Aβ. Three of 18 cognitively normal subjects were PIB positive and also had low CSF $A\beta_{1-42}$. These findings highlight the importance of combining PIB with other markers of AD progression to test the hypothesis that PIB-positive individuals who are cognitively normal or mildly impaired represent antecedent AD. Another recent study combined PIB PET with volumetric MRI. Archer et al. [44] found that whole-brain PIB binding correlated with rate of decline in whole-brain volume among a group of patients with a clinical diagnosis of AD. The authors concluded that amyloid deposition was associated with higher rates of atrophy, and that this supports a primary role for amyloid in AD. Two subjects in this study were identified clinically as AD, had very small change in brain volume, and were PIB negative; they did not show evidence of decline after 1 year and may have had a cause other than AD underlying their impairments.

Buckner et al. [56] have emphasized the correspondence between amyloid deposition and brain dysfunction as measured by FDG metabolism, and comparative studies of these PET tracers are underway. Klunk et al. [46] analyzed the relationship between PIB PET and FDG PET in AD patients and reported that selected neocortical association cortices, such as medial and lateral parietal and frontal, showed an inverse relationship between these measures, but other regions did not demonstrate a clear relationship. In addition, the dynamic range was lower with FDG [25••].

Conclusions

The future of amyloid imaging, in whatever form it eventually takes, will likely be strongly affected by whether and when antiamyloid therapies prove effective. The initial experience with antiamyloid immunotherapy (the AN-1792 trial), although halted because 6% of subjects developed a menengoencephalitis, was nevertheless encouraging because it indicated that this therapeutic approach was worth pursuing [18]. Amyloid imaging may be useful to confirm the presence of amyloidosis in subjects who are candidates for antiamyloid therapeutic trials, as well as a marker of drug efficacy. In addition, it is possible that a limited clinical role for amyloid imaging could be established in which certain individuals with questionable AD could undergo PET scanning to determine whether amyloid is present or absent as an indicator of likely underlying pathology.

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