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Biopsy Support for the Validity of Pittsburgh Compound B Positron Emission Tomography With a Twist

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Amyloid imaging with the positron emission tomography (PET) ligand Pittsburgh Compound B (PiB) has spread to many centers around the world since the presentation of its first human studies performed in Uppsala, Sweden, in 2002.¹ At the time of this writing, there are at least 17 sites in North America, 9 in Japan, 8 in Europe, 3 in Korea, and 1 in Australia that have successfully used PiB PET in thousands of subjects. Amyloid imaging with PiB has become an integral part of the Alzheimer's Disease Neuroimaging Initiative in North America and equivalent studies in Japan and Australia. The relatively rapid worldwide acceptance of this new technology lies partly in the potential utility of amyloid imaging for the following: (1) early (perhaps presymptomatic) diagnosis; (2) the development of anti-amyloid therapies; and (3) deepening our understanding of the pathogenesis of Alzheimer disease (AD). Another reason for the rapid dissemination of PiB imaging may have been the body of preclinical work supporting the concept that PiB retention accurately reflects the deposition of fibrillar β -amyloid (A β) deposits in the brain.^{2–4} That, along with the fact that the pattern of PiB retention in humans⁵ matched the expected pattern of amyloid deposition deduced from the study of postmortem brain tissue,^{6–8} built confidence in the validity of amyloid imaging and encouraged the proliferation of this technology. Confidence in the relationship between PiB retention and amyloid content has been further bolstered by 2 postmortem studies that correlated in vivo PiB retention to postmortem measures of amyloid deposition. The first of these focused on a case with a clinical diagnosis of dementia with Lewy bodies and extensive cerebral amyloid angiopathy.⁹ The second was a case that showed the typical clinical symptoms of AD and classic AD pathological findings at autopsy.¹⁰ These postmortem studies showed that the amount and location of in vivo PiB retention in these 2 cases corresponded very well to the amount and location of A β deposits present after death.

The study by Leinonen et al¹¹ adds one more facet to the correlation of in vivo PiB retention with brain A β pathological findings. The group took a unique approach to this correlation. Rather than focusing on patients who had received a PiB scan and following them up to autopsy, Leinonen and colleagues assessed a group of 125 subjects who had undergone brain biopsy as part of shunt placement for suspected normal-pressure hydrocephalus (NPH). Of these 125 subjects, 50 were identified as appropriate candidates for PiB PET studies and 10 of these agreed to participate. Apparently by chance, 4 subjects had no evidence of brain A β deposition, 1 had very slight evidence of diffuse amyloid, and 5 had fairly abundant A β deposits identified by the 4G8 antibody. It should be kept in mind that the 4G8 antibody recognizes a region in the middle of the A β peptide (residues 17–24) and has been shown by Spillantini et al to stain “mostly amyloid plaques without cores.”¹² Spillantini and colleagues also showed that certain antibodies directed against the carboxyl-terminal region of A β stain “only amyloid plaques

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with cores.”¹² Leinonen and colleagues mention that they also used the 6F/3D antibody and say that it is “reactive to amino acid residue 10–15”¹¹ and labels “both parenchymal aggregates (ie, plaques) as well as cerebral amyloid angiopathy.”¹¹ However, they report no 6F/3D data in their article. Although they included Bielschowsky silver staining and AT8 staining for hyperphosphorylated tau, the omission of staining with both N-terminal and C-terminal anti-A β antibodies as well as more classic amyloid fibril stains such as thioflavin S makes the presented data difficult to fully interpret, as we will see.

All of the 4 subjects without A β deposits showed no evidence of PiB retention in vivo. Three of these received a diagnosis of NPH and were apparently not demented (Clinical Dementia Rating Scale [CDR] score, 0–0.5); the other appeared to be demented (CDR score, 1; CDR sum of boxes [SOB], 6; Mini-Mental State Examination [MMSE] score, 22), did not receive a diagnosis of NPH, and was studied with PiB only 2 months after the biopsy. Thus, the cause of the cognitive change remains very unclear in their subject 4. Not surprisingly, subject 5, with trace deposits of fleecy A β aggregates, also showed no evidence of PiB retention. This person was cognitively impaired, had a CDR score of 1, CDR SOB of 2.5, and MMSE score of 24, and was diagnosed with NPH after the shunt.

Four of the 5 subjects (subjects 7–10) with histological evidence of moderate to heavy A β deposition showed the expected in vivo PiB retention. Subjects 7 and 8 were given a diagnosis of NPH and had relatively mild cognitive dysfunction, with CDR scores of 1 and 0.5, CDR SOBs of 3.5 and 1, and MMSE scores of 28 and 27, respectively. Thus, it is unclear to what degree the NPH and the AD pathological findings contribute to the cognitive impairment in these subjects. Subjects 9 and 10 were more severely impaired, with CDR scores of 2 and 1, CDR SOBs of 9 and 5, and MMSE scores of 22 and 23, respectively. They did not receive a diagnosis of NPH, leaving one to presume that the basis for their cognitive impairment may be AD. Despite the very small size of the frontal cortical biopsy specimens in these 4 subjects (2–5 mm in diameter and 3–7 mm in length), there was a good correlation between a semiquantitative measure of plaque count and in vivo PiB retention. Like the previous postmortem studies,^{9,10} these 9 subjects (subjects 1–5 and 7–10) further support the validity of PiB PET as an in vivo measure of A β deposition.

The most interesting aspect of the study by Leinonen and colleagues, however, may be the findings in subject 6, who showed histological evidence of A β deposition (very similar to that in subject 7) but no evidence of PiB retention 20 months after the biopsy. It is highly unlikely that the A β deposits present at the time of biopsy disappeared in the intervening 20 months before the PiB scan was performed given the stability of PiB retention that has been previously shown in studies of patients with AD.¹³ Therefore, it is important to understand the apparent failure of PiB PET imaging to detect these deposits. Leinonen and colleagues offer some possible explanations such as “potential technical errors of PET imaging”¹¹ that presumably refer to possible errors in production or administration of the PiB tracer or in acquisition of the emission data. Given the extensive experience of the Turku PET Centre and the quality-control steps used during PiB studies, one could assume that these are unlikely explanations. Another explanation offered by Leinonen and colleagues is an error in the “labeling of the histological samples.”¹¹ It is not unreasonable to consider this a possibility as many examples exist in the literature, but again, one must consider this unlikely. Leinonen and colleagues point out that the very small sample size of the frontal biopsy specimen could lead to either failure to sample A β deposits in other brain areas (false-negative biopsy) or sampling of a rare, unrepresentative area with very high levels of A β deposits (false-positive biopsy). One would think a false-negative biopsy would be much more likely than a false-positive one. There is no evidence of false-negative biopsies in this study (ie, A β -free biopsy and positive PiB scan results), and to my knowledge no examples of ultrafocal A β deposits have been reported in the literature to support a false-positive biopsy as an explanation of the finding in case 6. Leinonen and

colleagues briefly mention another possibility that seems the most likely scenario. They suggest “that there might be types of amyloid deposits that PiB does not detect.”¹¹ There is certainly precedence for this hypothesis. One glaring example of low PiB binding despite extensive A β deposits can be found in the experience with several types of transgenic mice.^{2,14,15} It has been clear from the beginning that, like thioflavin T itself from which PiB was derived, the binding of PiB to A β is highly dependent on the secondary and tertiary structures of the peptide. For example, PiB staining in postmortem tissue can be abolished by denaturing the β -sheet structure with formic acid.¹⁰ Maeda et al¹⁵ have suggested that PiB binding may be dependent on the presence of an N-terminally truncated and modified A β , A β N3-pyroglutamate—although this remains unproven. There also is evidence from human studies to suggest that PiB does not recognize all A β deposits equally. For example, PiB retention in patients with early-onset familial AD with certain presenilin 1 mutations is focally intense in the striatum but relatively low in the entire neocortex, even in subjects with clinical AD.¹⁶ Given that postmortem studies of the brains of mutation-carrying parents of these same patients with presenilin 1 mutations show a pattern of amyloid deposition typical of late-onset AD,¹⁷ it is certainly possible that there is more neocortical amyloid in these cases than PiB PET would suggest. A very recent case study by Tomiyama et al¹⁸ described a patient with early-onset familial AD with a novel amyloid precursor protein mutation lacking glutamate 22 (E22 Δ). This mutation leads to aggregation in a predominantly oligomeric form apparently lacking fibrils. Although Tomiyama and colleagues do not know whether their patient has any parenchymal A β deposits because they have no histological data, they note that the patient has relatively low PiB retention despite severe clinical dementia.¹⁸

Perhaps, then, subject 6 in the study by Leinonen and colleagues has a form of A β deposit that does not bind PiB well. Subject 6 is a clearly impaired 72-year-old patient with a CDR score of 1, CDR SOB of 5.5, and MMSE score of 19. A diagnosis of NPH was made based on the clinical evaluation and shunt results, so the relative contribution of NPH and AD pathological findings to the dementia is not clear. Interestingly, although they report 39 4G8-stained plaques per $\times 100$ field, the biopsy sample was devoid of AT8 staining for hyperphosphorylated tau and Bielschowsky staining showed no diffuse plaques and only 1 neuritic plaque per $\times 100$ field. While one might be tempted to suggest that the Bielschowsky stain accurately predicted the negative PiB scan result in subject 6, subject 7 had a very similar clinical and histological profile (42 4G8-stained plaques and only 2 diffuse plaques on Bielschowsky staining) but showed high PiB retention. It may be important to note that the other 3 cases with positive PiB scans had 45 to 80 4G8-stained plaques and 11 to 20 diffuse plaques on Bielschowsky staining. The 2 cases with the highest plaque load were AT8 positive and did not keep the diagnosis of NPH after the shunt procedure. It is unfortunate that we do not have more extensive histological data on these extremely important biopsy specimens. Further data using antibodies directed at other A β epitopes (including A β N3-pyroglutamate) as well as fibril stains such as thioflavin S or the highly sensitive Congo red derivative X-34¹⁹ could be very helpful in sorting out this apparent PiB false-negative result. Staining with the highly fluorescent PiB derivative 6-CN-PiB also could be very helpful.^{19,20} This case is an important example of how the use of PiB PET can deepen our understanding of the pathogenesis of AD and the biology of A β deposits.

Thus, although case 6 may represent the most fascinating aspect of this study, one must not lose perspective of the fact that PiB PET correctly identified 5 of 5 subjects with no (or trace) A β deposits and 4 of 5 subjects with significant A β deposits. This implies that it is highly likely that the increasing number of reports showing low to moderate PiB retention in cognitively normal control subjects are not false-positive PiB scan results but identify subjects with significant A β deposition.^{5,21–23} However, case 6 raises the possibility that a negative PiB scan result could be obtained in the presence of atypical amyloid deposits. Unfortunately, we cannot be certain of this possibility until there is a full histological evaluation of a case such as this, and that will require a full postmortem examination.

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