Toronto: Ah, The Devil in the Details

This is Part 2 of a six-part series. See also .

5 May 2010. At the 4th Human Amyloid Imaging Conference held on 9 April 2010 in Toronto, several themes generated intense discussion. On the technical side, the question kept cropping up of what is the right reference region to use in amyloid imaging. Typically, imagers compare any given region's amyloid burden to that of cerebellum, because this brain area remains relatively unscathed deep into AD progression. Trouble is, it doesn't always. In some studies, some parts of the cerebellum do show some amyloid. For example, this is the case in autosomal-dominant forms of AD and must be taken into account for amyloid imaging studies of the DIAN, noted **Stephen Salloway** of Butler Hospital in Providence, Rhode Island. In fact, at HAI, scientists led by **David Brooks** at Hammersmith Hospital in London presented first PIB imaging data on seven presenilin-1 mutation carriers along with those of 10 sporadic cases and 10 controls. (These are U.K. families in the care of **Martin Rossor** and **Nick Fox** at University College, London.) This study indeed found increased cerebellar uptake in some mutation carriers.

Some studies have bracketed off amyloid-containing sub-areas of the cerebellum and use the rest as the reference region, while other studies use the entire cerebellum. If scientists optimize the reference region they choose, they can make their data look stronger by widening the separation between amyloid-positive and amyloid-negative groups. At a different conference last month in Geneva, **Eric Reiman** of the Banner Alzheimer's Institute in Phoenix, Arizona, illustrated this point. He showed on a slide how comparing a given dataset against a reference region drawn from different parts of the cerebellum shifted uptake values for the brain regions of interest considerably.

In Toronto, several speakers emphasized the need to find consensus not only on which reference region to use but, even more so, on precisely how to delineate it on the brain atlas. This delineation is important because it affects the threshold above which a person is judged to be amyloid-positive. Different groups at present draw this cutoff in different ways, making comparisons difficult. "The cutoffs are a bit fuzzy," said **Val Lowe** of Mayo Clinic in Rochester, Minnesota. "We are not always comparing apples to apples yet. To do that, we need to agree on what reference region to use," agreed **Jessica Langbaum**, also at Banner. Other scientists cautioned that shrinkage over time of the cerebellum could introduce error into longitudinal studies, requiring its own correction. Atrophy in general is a bit of a puzzle to amyloid imagers. They don't know if a person loses plaque as they lose brain tissue, or if the plaques stay and become more concentrated, noted **Bill Klunk** of the University of Pittsburgh, Pennsylvania.

Scientists led by Brooks presented two posters describing how they compared the cerebellum's and the pons's discriminatory power as reference regions for a PIB and an 18F flutemetamol dataset (18F flutemetamol is a ligand developed by GE Healthcare). They concluded the pons is suitable in studies where cerebellum is not, but discussion reached no general consensus on the issue. Other scientists, for example, **Osama Sabri**

from the University of Leipzig, Germany, showed data supporting the use of the cerebellar cortex for reference.

Beyond normalization, technical debate also revolved around the methods by which neuroimagers analyze the raw data in order to quantify the amyloid in a person's brain. For example, scientists discussed the relative merits of a method called distribution volume ratio (DVR) versus one called standard uptake value ratio (SUVR). Without getting overly arcane in a news story, the gist of the argument is that the DVR method is generally considered to be more sensitive, but as the field is expanding, a growing number of investigators use only the simpler SUVR method. DVR requires a data acquisition lasting 60-90 minutes while the PET camera makes a movie of about 50 frames. The SUVR comes from an image that is summed over 10-30 minutes, so the participant lies in the scanner for a shorter period of time.

At HAI, **Ann Cohen** from the University of Pittsburgh evaluated both methods side-by-side in 62 cognitively normal controls. The question was not whether the SUVR can distinguish garden-variety AD cases from controls—by general consensus, it can—but how sensitive it is in picking up small amounts of amyloid in people whose levels might hover right around a threshold of amyloid positivity. For these people, subtle differences in the analysis method could well determine on which side of such a cutoff they end up, hence, changing a study's result. Cohen reported that the DVR produced data within a narrower range than the SUVR method and classified three people as amyloid-positive whom the SUVR did not pick up as such. **Kenji Ishii** of the Tokyo Metropolitan Institute of Gerontology in Japan, in presenting the first amyloid imaging results from the J-ADNI study (see Part 1 of this series), concurred, saying that DVR analysis showed considerable amyloid deposition in three of 33 people classified as negative by the SUVR. Others disagreed. Sabri noted that his proof-of-mechanism study of the Bayer Healthcare F18 compound florbetaben found no such difference.

In fact, sensitivity was a sensitive subject throughout the day. DVR versus SUVR represents but one aspect of it; another is whether to measure amyloid in the brain globally or by region. For her part, Cohen reported that among the 62 volunteers, regional PIB values classified a greater number as amyloid-positive than did global PIB values. She suggested that a global analysis does a fine job of analyzing widely distributed amyloid deposition, but not of measuring the first indication of amyloid in any brain region. **Elizabeth Mormino** of the University of California, Berkeley, agreed, saying that in her hands, too, the earliest increases in amyloid most reliably lit up in certain local regions, above all the precuneus. For his part, **Keith Johnson** of Massachusetts General Hospital, Boston, suggested that this distinction is scientifically interesting beyond the immediate goal of getting amyloid imaging ligands approved. "In terms of detecting amyloid, we have learned something in the past year. All of us have seen cases where there is highly focused uptake in small regions, and we follow these people and see it spread from there. I hypothesize that these people have a sea of prefibrillar amyloid in their brain, and certain areas 'poke through' with fibrillar deposition and reveal themselves. In detecting amyloid, which is the FDA requirement we are all thinking

about, is it important to recognize these biological features, or just the instrumental features we are talking about?"

Similarly, Klunk recommended that investigators stay acutely aware of the sensitivity of the methods they choose. "We are beginning to see amyloid-negative cases with neuropathologically evident amyloid, suggesting that the threshold we use to detect reflects a fair amount of amyloid in the brain. That is true even with the most sensitive DVR measures. If we miss more by using SUVR to measure amyloid, do so globally, and miss even more by using an F18 ligand with higher white matter staining, then we may end up missing quite a lot." Scientists said this conundrum represents the tension between scientific accuracy and the practical and financial constraints of broadly applicable multicenter protocols. In short, investigators need to select their methods based on the sensitivity needed for each particular study.

One good example for the importance of analytic sensitivity is this key research question: What is a positive PIB scan? "Answering this simple question is not straightforward at all," said Mormino. In the past two years, one of the greatest surprises in the field has been the large proportion of cognitively normal people who have significant amounts of amyloid in their brains. And yet, defining just from what point on a PIB scan counts as positive is tricky. At HAI, Mormino presented a new study to get at this problem. She compared two methods of establishing a threshold—one a previously published objective approach that removes outliers from among elderly control data (Aizenstein et al., 2008), and a subjective, simple approach of cutting at two standard deviations above the mean PIB value she'd measured in seven twentysomething controls. Mormino then applied the cutoffs derived in these two ways to 52 old controls and 25 AD patients.

The result? Both methods classified all AD patients as PIB-positive. Among the elderly controls, the Aizenstein method proved more conservative, putting eight of 52 into the PIB-positive bucket versus 15 as per the subjective approach. These eight folks are probably truly positive, validating this approach to creating a cutoff, Mormino said. They also tend to be older, more likely to have ApoE4, have smaller hippocampi and worse episodic memory than the people in the PIB-negative group; hence, their profile fits a preclinical AD picture. The seven volunteers that came out as PIB-positive by one approach but not the other form an interesting group that warrants longitudinal follow-up with imaging and other preclinical measures, Mormino said. Their signal could be noise, or it could be biologically meaningful, representing an earlier stage of amyloid accumulation. As to sensitivity, for this study Mormino chose PIB as the ligand and DVR as the analysis method.

HAI attendees agreed that it is important to forge some agreement around exactly what constitutes amyloid-positive, and also, what constitutes cognitively normal. Why? These definitions will influence much future research. For example, researchers are beginning to experiment with modeling PIB data from cognitively normal people to generate initial incidence and prevalence estimates. **Mark Mintun** showed how that could work with data from volunteers at Washington University, St. Louis. He ran longitudinal PIB-PET scans on 129 people age 45 to 88, set a threshold, and calculated how quickly people

crossed it. From there, Mintun computed an incidence rate of 2.9 percent of adults becoming amyloid-positive per year. The prevalence of having plaques in the brain came out as increasing from 4.4 percent in one's fifties to 14 percent in the sixties and 50 percent in one's nineties. Mintun calculated a delay between amyloid plaques and dementia of about a decade. This points to a future epidemiology of brain amyloid in the normal population. Yet in these early days, scientists operate with small datasets and with assumptions that vary somewhat from site to site; hence, their results also vary from site to site.

Illustrating this, **Chris Rowe** of Austin Hospital in Melbourne, offered the perspective from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging, which also supports epidemiological calculations (Ellis et al., 2009). His calculation of delay between plaques and dementia came out closer to 20 years. In general, Rowe had even more "depressing" numbers, as he called them. "In our hands, the prevalence of amyloid positivity in the sixties is 50 percent. We have plenty of old people in the study, and it looks as if amyloid shows up in everyone if you live long enough," Rowe said. In discussion, suggestions for this extreme variation in the numbers ranged from the facetious ("watch that kangaroo meat—maybe it's amyloidogenic") to the serious. The argument that echoed throughout the day was that results depend on how the "normal" group is being assembled. In the WashU study, participants are assessed every year and moved out of the "normal" pool as soon as they show deficits, whereas in the AIBL study, the normal cohort might contain more people with mild impairments, some said. **Bill Jagust** of the University of California, Berkeley, concluded the topic with a call on neuroimagers everywhere to insist on careful characterization of the normal cohort in their future studies.—Gabrielle Strobel.

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