Investigating in vivo Imaging of Alzheimer's Through Immunohistochemical Analysis

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IN-VIVO IMAGING OF ALZHEIMER'S

As of right now, there is no reliable tests available to…

1. Provide **Definitive Diagnosis** for Alzheimer's Disease (AD) during life. All definitive tests require neuropathologic examination of brain tissue, conducted almost exclusively postmortem.
2. Track pathogenic response to **drug intervention** in human clinical trials
3. Determine presence of AD in its early stages, where **treatment** would be most helpful.

HOWEVER, an *in-vivo* test for AD may be able to solve these issues.

NIR IMAGING'S POTENTIAL

What it does

◊ A new technique, outlined in the study *Scattering differentiates Alzheimer disease in vitro* and developed by Dr. Eugene Hanlon and his associates, leverages the optical properties of AD brain tissue to differentiate AD brains from non-demented brains.

◊ Utilizes Near-Infrared Light (NIR), a harmless light frequency between 600 and 900nm, deemed favorable for *in vivo* imaging due to relatively low tissue absorption and negligible autofluorescence.

◊ Technique has proved effective in *in vitro* setting, and shows promise in an *in vivo* setting

How it works:

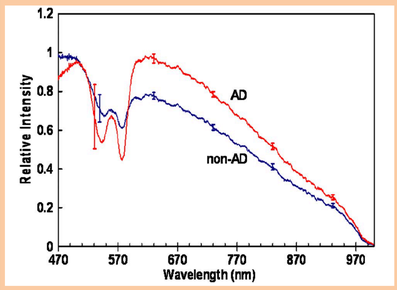

Figure 1. Mean diffuse reflectance spectra of intact temporal pole specimens from neuropathologically confirmed AD cases (AD), n=5, and control cases (non-AD), n=4. Error bars indicate the standard error of the mean for the AD and non-AD populations based on the spectra measured for each group." (Hanlon et. al., 2008)

THE PRESENT STUDY: VERIFICATION OF NIR IMAGING

Primary Objective: Determine which features of AD brain tissue cause this heightened diffraction. *In order to…*

1. Determine the range of uses of NIR imaging
2. Investigate potential complications in NIR imaging
3. Determine if NIR imaging can be used to detect early-stage or pre-symptomatic AD (if pre-symptomatic biomarkers do, in fact, cause diffraction)
4. Provide data that may help calibrate the NIR imaging technique

Overview: Prior to this study, NIR had been tested in vivo on 5 living AD patients. In this study, we conduct tissue analysis on the post-autopsy temporal lobes of these 5 patients and will eventually compare our data with Hanlon's.

Goals of Our Experiment:

1. Perform Tissue analysis through Immunohistochemistry— stain for key AD biomarkers including hyperphosphorylated tau (p-tau), amyloid beta (Aß), and potentially other biomarkers as well.
2. Conduct stereology in order to estimate number of and volume occupied by key AD biomarkers
3. Compare the present study's results with the results obtained in *Scattering differentiates Alzheimer disease in vitro*
4. Look for correlations.

Currently , this experiment's ***final results are pending.*** The lab group is currently still running immunohistochemistry on the AD tissue. They will perform stereology after completing this phase and will then begin forming correlations and comparisons between NIR data and the stereological data.

HYPOTHESIS:

We hypothesize that light diffraction readings will correlate with either

1. The number of amyloid beta plaques
2. The number of hyperphosphorylated tau structures such as neurofibrillary tangles and neuropil threads
3. OR… A combination of these Alzheimer's biomarkers.

MATERIALS AND METHODS

Immunohistochemistry (IHC)— A method used to visualize the distribution and localization of specific cellular components within cells and in the proper tissue context through antigen-antibody binding.

Our Strategy:

We choose to use the ABC (for avidin-biotin-complex) peroxidase method for IHC with diaminobenzidine (DAB) as our substrate. Currently our analysis is focused on p-tau and Aß, two of the most prominent microscopic features in AD.

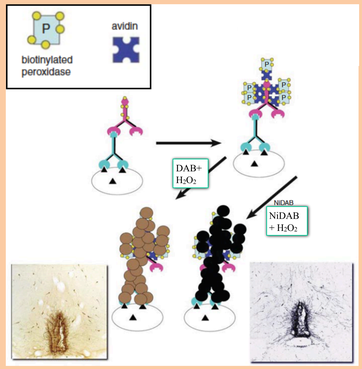

Figure 2. The Process of ABC peroxidase IHC involves first binding a primary antibody to the antigen of interest, then binding a secondary antibody to the primary, and finally binding avadin to the secondary antibodies. A reaction is then induced in which biotinylated peroxidase binds to the secondary antibody, serving to significantly amplify the staining signal. The tissue is then incubated in the substrate (DAB and Nickel DAB are the two dyes shown in this graphic) and a dying product is generated.

Next Step:

After performing IHC, my lab group will conduct **stereology**— a method which allows for three-dimensional interpretation of two-dimensional cross sections of tissues— to obtain quantitative data on the volume, area, and number of tau and amyloid structures. This will allow us to compare, contrast, and form correlations between our data and Hanlon's data.

ONGOING DATA COLLECTION

Target 1: Tau

Tau proteins are microtubule (MT) proteins purposed to stabilize MTs. In AD, however, these proteins are abnormally phosphorylated, resulting in large tangles of hyperphosphorylated-tau (p-tau). These tangles, referred to as neurofibrillary tangles (NFTs) are large enough and plentiful enough to cause a high degree of light scatter.

Tau is, therefore, a primary target in our IHC. We used antibody AT8 for p-tau staining. Additionally, we targeted p-tau typically seen in early-stage AD by staining with antibody CP13.

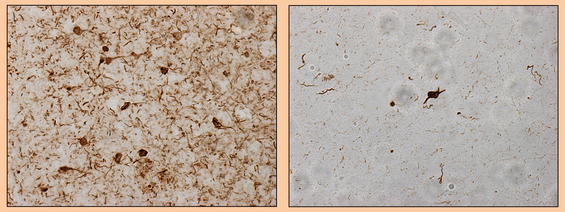

Figure 3: Stained tissue sections shown from the temporal lobes of two of the 5 AD patients in our study. Immunostaining was done with tau antibody AT8, and diaminobenzidine (DAB) was used as the substrate. The large collections of p-tau resembling cell-bodies are Neurofibrillary tangles (NFTs) whereas the smaller, string-like strains of tau are known as d (NPs). Both slides depict immunostaining with AT8 at 1:200 dilution and 200x magnification. Images courtesy of Dr. Douglas Rosene, Dept. of Anatomy & Neurobiology at Boston University School of Medicine

Target 2: Amyloid beta

Amyloid Beta (Aβ) plaques are protein accumulations found extracellularly in AD brain tissue. Aβ plaques are relatively large and plentiful in most Alzheimer's cases, especially in late-stage AD. Due to their prevalence in AD and their likelihood of having a high refractive index, these plaque are also of utmost interest to our research group.

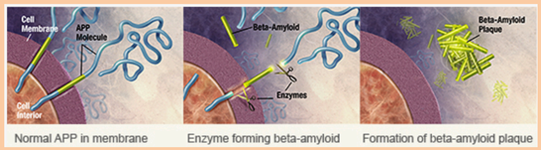

Figure 4: In Alzheimers disease, Aβ plaques form when enzymes "cut" the Amyloid Precursor Protein, thus exposing the Aβ peptides. These peptides stick together, forming large plaques. Illustration Credit: National Institute on Aging, National Institutes of Health

DISCUSSION

After conducting stereology, the lab group will attempt to find a correlation between Hanlon's diffraction data and one or both of the aforementioned AD biomarkers. The nature of the results we obtain will have major implications on the future of NIR Imaging…

1. P-Tau– If p-tau is found to be the primary cause of light scatter:

◊ NIR imaging's ability to test for the presence of AD during symptomatic stages would be further validated ➡ *P-Tau is present in virtually all AD cases*

◊ NIR imaging may prove functional in estimating the degree of cognitive decline present in each AD case ➡ *Number of NFTs in brain tissue is considered a reliable estimate of cognitive decline*

◊ If early stage p-tau is *also* determined to cause light-scatter, NIR could potentially detect AD *before* it presents clinically

◊ NIR could track how tau responds to drug intervention

2. Aβ– If Aβ is found to be the primary cause of light scatter in AD:

◊ NIR imaging will still likely be a reliable candidate for disease detection during symptomatic stages ➡ *Though it varies from case to case in quantity, Aβ is present in virtually all AD cases by the time symptoms appear.*

◊ *HOWEVER*, NIR would be significantly less accurate at estimating cognitive decline ➡ *Quantity NFTs has been shown to be a far better predictor of cognitive decline.*

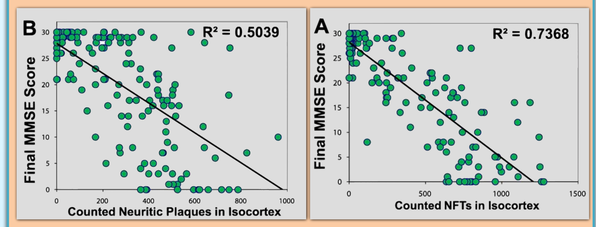

Figure 5: The above data from the Journal of Neuropathology and Experimental Neurology (2007:66:1136–46) demonstrates the correlation between antemortem cognitive status (final Mini-Mental State Examination [MMSE] scores) and NFT count (left) or Neuritic Plaque count (right). 178 patients without concomitant neuropathologic findings were sampled. Each circle represents data from a single individual. NFTs and NPs were counted and summed from 4 different portions of cerebral neocortex: Brodmann areas 21/22, 18/19, 9, and 35.

3. Both— If both Aβ and p-tau contribute to heightened light scatter

NIR Imaging technique will still be a reliable candidate for AD detection, since both biomarkers indicate AD presence. NIR may also be able to estimate cognitive decline; however, this estimate may be complicated by the variability of AB quantity from case to case.

POTENTIAL FUTURE RESEARCH:

- Determine if NIR imaging is also sensitive to other taupathies or neurodegenerative diseases (a potential complication)
- If neither of the investigated biomarkers correlate with light scatter… Determining if other biomarkers/ macroscopic tissue features account for heightened light scatter in AD cases.
- Further investigation into relationship between light scatter and cognitive decline
- Further investigation into whether or not NIR imaging can pick up on pre-symptomatic biomarkers in AD

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