Current status in Alzheimer's disease biomarkers : a literature review and critical summary of the field

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Dedication

I dedicate this paper to my husband for his support through the entire journey of physician assistant school; for helping at home while school and clinical rotations demanded all of my time, and for giving me confidence to fulfill my dream.

To my sister Gretchen—without her encouragement, I would not have looked into a career as a PA, nor would I be the person I am today.

And for those living with Alzheimer's disease, for those whose loved ones live with Alzheimer's disease, and for those who have yet to be diagnosed, I dedicate my time and effort put into this project to moving closer to a better future for you.

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Introduction

More than forty million people are currently living with Alzheimer's disease (AD), and that number is expected to quadruple by 2050 (Ortiz, 2015). AD is a prevalent disease without a cure and only mildly efficacious treatment. At this time, funding and research is focused on several different aspects of AD (pathophysiology, diagnostic methods, pharmacological treatment, non-pharmacological interventions, etcetera), with the notion that if a definitive cause can be discovered, the rest of the puzzle that is Alzheimer's disease, will be solved. Although researchers have created a few well-supported theories about AD pathophysiology, still remains the missing link between these all of these principles. It is well known that the brain of people with living with Alzheimer's disease is riddled with amyloid beta (Aβ) protein plaques and tau protein tangles, but the etiology of these findings is still an unknown. And, currently, the only definitive diagnosis is made *post mortem* via brain tissue analysis for Aβ plaques and tau protein tangles. Through research, several factors have been suggested to be contributory in the development of these protein accumulations, such as "age, female gender, low educational and occupational attainment, prior head injury, sleep disorder (e.g., sleep apnea), estrogen replacement therapy, and vascular risk factors…" (DeFina, 2013), but nothing has been confirmed.

While some researchers are focused on the causes of AD, others are looking at the pathophysiology of disease, and what each person with Alzheimer's disease has in common. In determining a consistency across this population, a definitive diagnosis of AD can be made. The current gold standard of the diagnosis of Alzheimer's disease is made through clinical presentation—taking into the consideration the past medical history, family history, current symptoms, physical exam, and cognitive function. Currently the diagnostic accuracy is "only

approximately 70% even in most experienced medical centers," (Yu, 2016). In addition to a clinical diagnosis, clinicians are able to support their diagnosis with measurement of the tau and Aβ in the cerebrospinal fluid, visualizing the amyloid burden in the brain via positron emission tomography (PET) scan, and assessing the level of brain atrophy as seen on a magnetic resonance image (MRI) (Yu, 2016). Cognitive function can be assessed via the standardized Mini-Mental State Exam (Figure A). This, however, can produce subjective results according to a patient's baseline educational status, the person proctoring the exam, and the patient's overall well-being that day.

Clinical diagnosis is further supported by the presence of amyloid β (Aβ), total tau (t-tau), and phosphorylated tau (p-tau) in the cerebrospinal fluid (Leung, 2015). These biomarker proteins have become an accepted tool by which other biomarkers are measured due to their proven consistency in the presentation of patients with clinically diagnosed Alzheimer's disease. One weakness with cerebrospinal fluid (CSF) Aβ is that it has been shown to have "low correlation with cognitive symptoms," (Leung). Another is that t-tau does not "track cognitive changes closely [enough]" (Leung) for effective monitoring. Herein lies the need for other biomarkers that can definitively diagnose AD with great specificity, and allow clinicians to monitor changes with treatment. If biomarkers from CSF could be shown to have distinct specificity and accurate sensitivity to the diagnosis of AD, the ability to diagnose the disease would allow for: (a.) More accurately selected cohorts for testing potential AD treatment options, (b.) Determination if treatment needs to be initiated, or (c.) For effective monitoring of the progress of patients being treated (Leung). Aside from the clinical aspect, there is a mental peace in having a diagnosis for a collection of symptoms so that non-pharmacological treatment and arrangements can be created or implemented to better the lifestyle of someone living with AD.

Aside from biomarkers in the CSF, biomarkers from blood plasma can also be measured and utilized. Researchers have also been able to narrow the genome to multiple genes shown to somehow correlate to AD or affect pathology related to AD. Another option is focusing on the degenerative changes seen in the brain of patients with Alzheimer's disease. Through magnetic resonance imaging (MRI), the gray and white matter can be studied and quantitatively measured. However, while MRI is relatively cost-effective (compared to other imaging modalities utilized in neuroimaging), has low radiation exposure, and is widely available, the sensitivity of imaging typical-sized plaques is limited as the resolution is significantly more poor than other imaging options (Tong, 2015). Other modalities include using radio-labeled positron emission tomography (PET) and single photon emission computed tomography (SPECT) probes, however, these methods are expensive, have limited availability, and cause increased exposure to radiation. An option that warrants further research is the use of near-infrared (NIR) probe fluorescence, as it is nonradioactive, rather inexpensive, and widely available (Tong).

No matter what the method may be, diagnosing Alzheimer's disease is imperative to the ability of researchers to find a cure, or at least better treatment options, for those patients living with AD. Although there are a variety of approaches, finding an appropriate technique that is not only cost-effective, non-invasive, and widely available, but also accurate and specific to AD, is proving to be a significant challenge. However, with all the work that researchers are currently conducting, the expectation that one of these avenues is *the* one that will lead to a definitive diagnosis, is not out of the realm of possibility.

Literature Review

Mini-Mental Status Exam for the detection of dementia. This is one of the first, if not the first, screening tool for dementia in symptomatic patients. Could this screening tool be integrated into the annual physical exam as a way to catch AD early or earlier? Because older patients are more likely ignore signs of AD and blame them on their age, perhaps making the MMSE a routine part of their physical will highlight pathologic problems, leading to an earlier diagnosis.

Cerebral spinal fluid biomarkers. Examples include: neurogranin, YKL-40, amyloid beta, total tau and phosphorylated tau, and TREM2. Levels of these proteins have been shown to correlate with the clinical presentation of patients with AD.

Biomarkers in DNA detected from blood samples. Examples include methylation in the NCAPH2/LMF2 promoter region and methylation in APOE genotype. Measuring levels of VEGF and soluble CD40L levels in the serum have been suggestive diagnostic measures for AD.

Specific genes have been linked to AD. Apolipoprotein E **(**APOE) ε4 gene allele has been proven to correlate with an increased risk of AD. Currently researchers are still searching for a gene that can be definitely shown to either cause Alzheimer's disease or provide a definitive diagnosis. The gene coding for TREM2 has been correlated with possible increased risk of AD.

Using biomarkers to visualize neuronal changes due to AD. Near-infrared fluorescent probes for imaging amyloid plaques have been developed—discuss pros and cons (cost, availability, safety, etc.) of this method of imaging.

Using magnetic resonance imaging (MRI) or positron emission tomography (PET) scans to diagnose patients with AD. MRI can be used to compare the hippocampal texture in "healthy" patients versus those with AD. PET scans with radiotracers and fluorescence indicators have been most recently discussed as effective diagnostic methods for AD.

Discussion

Cerebrospinal Fluid Biomarkers

Cerebrospinal fluid (CSF) is obtained via a relatively cost-effective and non-invasive method, allowing for somewhat easy sampling that can be collected at nearly any institution. Over the past two decades, researchers have been attempting to narrow certain proteins as markers correlating with Alzheimer's disease (AD) to aid in defining a definite diagnosis in the disease. Currently there are three major proteins detectable in CSF used to support a clinical diagnosis of AD: increased total tau (T-tau) and phospha-tau (P-tau), and decreased levels of 42 amino acid-long, aggregation-prone Aβ protein (Aβ42) in the CSF—these proteins are called the "core AD biomarkers" (Heslegrave, 2016). Newer protein markers that have been reflective of AD symptoms (cognitive decline and memory impairment) are compared to these "core biomarkers" to measure their efficacy (Heslegrave). One example of this method is through the testing of neurogranin and YKL-40.

Neurogranin[1] is a marker of synaptic degeneration, and YKL-40[2] is a marker of neuroinflammation (Janelidze, 2015). Both of these pathologic processes are thought to be contributory to the symptoms seen in Alzheimer's disease. In a study conducted by Shorena Janelidze et al., 388 individuals with a variety of severity of neurological diseases[3][4], as well as

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[1] Neurogranin is a "calmodulin-binding postsynaptic protein regulating synaptic plasticity and learning." (Janelidze, 2015).
[2] YKL-40 (chitinase-3 like-1, cartilage glycoprotein-39) is a "potential marker of ongoing inflammations in a variety of human diseases" (Janelidze, 2015).
[3] Neurological diseases included "patients with stable mild cognitive impairment (sMCI), MCI(who later developed AD (MCI-AD), AD dementia, Parkinson's dementia (PDD), dementia with Lewy Bodies (DLB), vascular dementia (VaD), and frontotemporal dementia (FTD)," (Janelidze, 2015).
[4] All patients were clinically diagnosed with dementia according to the "DSM-IIIR criteria for dementia, combined with with NINCDS-ADRDA criteria for AD, the NINDS-AIREN criteria for VaD, [and] criteria for probable DLB," (Janelidze, 2015)

"cognitively healthy controls" were compared, via a CSF sample, to measure the level of neurogranin and YKL-40 in their CSF—these values were then cross-compared to the core AD biomarkers. Levels of CSF neurogranin were measured using an "in-house sandwich enzyme-linked immunosorbent (ELISA) assay," YKL-40 levels were measured via commercially available ELISA kit, and the core AD biomarkers were analyzed using Euroimmun immunoassay (EUROIMMUN AG) (Janelidze). The results were compared across the different diagnosed dementia types (Figure B).

Additional studies have shown that levels of neurogranin are reduced in the brain but elevated in the CSF in AD patients; concurrently, YKL-40 levels are elevated in the CSF of AD patients (Janelidze, 2015). However, YKL-40 levels have not been proven to have the ability to diagnose patients with AD as levels also appear to be elevated in clinically normal patients as they progress in age, in addition to those with preclinical AD, vascular dementia, and frontotemporal dementia (Janelidze). Ultimately, researchers concluded that the "diagnostic accuracy of the Aβ42/YKL-40 was not improved compared with the Aβ42/ Aβ40 and Aβ42/tau ratios when differentiating patients with AD dementia from those with non-AD dementias," (Janelidze). These biomarkers correlate with the core AD biomarkers' levels, but do not distinguish between the different forms of dementia better than these other proteins.

According to Janelidze et al. (2015), the ratios of CSF Aβ42 to Aβ40 or tau have "a greater diagnostic accuracy in AD and show improved concordance with amyloid positron emission tomography (PET) imaging." Because neither the ratios Aβ42/neurogranin nor the Aβ42/YKL-40 were more accurate than the previously discovered ratios in differentiating the various dementia types, these proteins "do not provide any added clinical diagnostic value to already existing AD biomarkers during prodromal and dementia stages" (Janelidze).

The purpose of this study was to find biomarkers that could distinguish between the various types of dementia accurately, as well as, aid in making that diagnosis earlier in the disease process (Janelidze, 2015). These two goals are imperative in helping clinical trials of potential treatments accurately test the efficacy of the proposed medications, as well as reduce the costs and failure rates of the clinical trials. If the patients are misdiagnosed with AD, or if AD is diagnosed too late into the progression of the disease, medications are going to have a limited ability in actually altering or treating the symptoms, and therefore will not accurately assess the drug as a potential care options for Alzheimer's patients. Additionally, if biomarkers for AD could be distinguished, they could potentially aid in monitoring the progression of the disease.

One component of the pathogenesis of Alzheimer's disease is an inflammatory reaction that advances the progression of the damage to the brain. Researchers Heslegrave et al. (21016) have located a gene that, when mutated, codes for a protein likely involved in that inflammatory response: *TREM2*. A heterozygous mutation in the in *TREM2* gene has "been identified as [a] risk factor for Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and FTD," (Heslegrave). The resulting protein, TREM2, can be measured in an affected patient's CSF (Heslegrave). TREM2 is a receptor glycoprotein expressed on myeloid cells that is part of the immunoglobulin "superfamily," (Heslegrave). It has been shown to promote phagocytosis, suppress inflammatory cytokine production and enhance anti-inflammatory cytokine transcription in *in vitro* (Heslegrave). Because of this protein's role in the inflammatory process, a mutation that affects the functionality of the protein, could result in uncontrolled inflammation (Heslegrave). As TREM2 also has probable phagocytic properties of amyloid β (Aβ), dysfunction could result in abnormal accumulation of these proteins—as seen in Aβ plaques in Alzheimer's disease patients.

For this study, Heslegrave et al. (2016) collected a cohort consisting of 37 patients clinically diagnosed with AD (which was supported with a positive AD biomarker profile of tau and Aβ pathology), as well as 22 control individuals "who were cognitively normal and had a negative CSF AD biomarker profile." What the researchers found was that the patients diagnosed with AD had significantly higher concentrations of CSF sTREM2 than those the control patients, and these levels correlated with the patients P-tau and T-tau, as well as YKL-40, but not Aβ or MMSE scores (Heslegrave). In regards to *APOE ε4*, CSF sTREM2 concentrations were similar in those diagnosed AD patients with and without the gene (Heslegrave).

These results (Figure C) suggest that TREM2 could be involved in the pathogenesis of AD, but the exact mechanism is still unknown (Heslegrave, 2016). The CSF concentrations are consistent in patients living with Alzheimer's disease, despite the status of *APOE ε4* gene, which makes the testing more sensitive for AD. However, Heslegrave et al. noted that because the levels of sTREM2 were elevated in patients with other neurodegenerative diseases, the specificity to AD could not be determined. Because the CSF levels of sTREM2 positively correlated to YKL-40[5], as well as more mildly positively correlated to CCL2, the conclusion that sTREM2 is involved in microglial activation is further supported (Heslegrave). Because the CSF Aβ levels show no relation to the CSF sTREM2 levels, and sTREM2 is involved in the inflammatory pathology associated with AD, it is suggested that Aβ plaque deposition occurs "independent[ly] of inflammatory processes" and the pathogenesis resulting from these plaques might affect a different cascade of neurodegeneration (Heslegrave). Further testing is necessary to determine the usefulness of this biomarker—while it may not be helpful in diagnosing AD,

[5] YKL-40 and CCL2 are CSF biomarkers that have been shown to correlate with clinically diagnosed AD, although they are not specific to AD, alone (Janelidze, 2015)

CSF levels of sTREM could potentially allow for monitoring of the therapeutic interventions for future treatment options (Heslegrave).

Blood-based Biomarkers

When creating a biomarker, several factors must be evaluated to determine if it is going to function efficaciously. With blood-based biomarkers, such as VEGF and soluble CD40L, the benefits have proven this method to be worthy of much research. Not only are blood-based biomarkers non-invasive, relatively inexpensive, and time-effective, but they have also been shown great specificity and sensitivity to Alzheimer's disease (Yu, 2016).

Through the blood plasma, proteins are isolated and quantified, then compared to clinical diagnosis to determine the validity of the measurement (Yu, 2016). Shu Yu et al. conducted such an experiment, in which they measured the vascular endothelial growth factor (VEGF) and soluble CD40 ligand (sCD40L) levels in the blood serum of 90 patients (50 diagnosed with AD, 40 controls). Soluble CD40L and CD40 ligand interact with CD40 "resulting in pro-inflammatory and prothrombotic responses," as well as "produce many angiogenesis-associated factors, such as [VEGF]," (Yu). According to Yu et al., it has also been suggested that CD40 can induce Aβ production. In addition to its involvement in angiogenesis, VEGF as promotes vascular remodeling and endothelial blood-brain barrier maintenance. Recent studies have also shown that VEGF could potentially be an important component n the neurogenesis of the adult hippocampus[6] (Yu).

The data from the study suggested that these biomarkers could be valuable diagnostic biomarkers for AD either alone or when used in combination with a multiple-marker panel. The

[6] The hippocampus is the area of the brain that is important for learning and memory. It is a portion of the brain often examined in diagnostic imaging studies when looking at the brains of potential or diagnosed AD patients (Sorensen, 2016).

results, shown in detail in Figure D, indicate correlation between the VEGF and sCD40L levels and AD versus control patients. Additionally, the Log_{10}-transformed CD40 ligand (Lg(sCD40L)) and Log_{10}-transformed VEGF (Lg(VEGF)) were significantly increased in AD cases, and showed "significant and moderately strong positive correlation," (Yu, 2016). These values proved both great specificity and sensitivity (see Figure E) in regards to the diagnosis of AD (Yu). At this time, there is "no single blood-based biomarker that achieves sensitivity or specificity above 80%," (Yu).

The mechanism by which these proteins are linked to AD is not entirely clear, however there are several hypotheses related to angiogenesis and increased inflammation to areas with increased blood flow (Yu, 2016). Since one suggested pathogenesis of AD is due to inflammation of the brain tissue, an increase in the mode by which inflammatory cells reach various tissues, could suggest a possible correlation between the problem and the cause. In addition to a lack of a definitive route through pathophysiology, these proteins have also been shown to be elevated in other common pathologic diseases such as, myeloproliferative neoplasms, certain solid tumors, stroke, mild cognitive impairment (MCI), vascular dementia, and other neurodegenerative processes. Because of these findings, other causes for elevated sCD40L and VEGF must be ruled out before the levels can aid in the diagnosis of AD.

Despite the weaknesses in this study, measuring the serum values of CD40L and VEGF have still been probable add-on biomarkers in supporting a diagnosis of AD, and could potentially aid in early diagnosis in these patients.

Gene-Specific Biomarkers

In a study conducted by Kobayashi et al. (2016), another form of biomarkers were examined in an attempt to find a way to definitively diagnose, and diagnose earlier in the disease

progression, Alzheimer's disease. As APOEε4 genotype is regarded as an indicator for increased risk of developing AD, it was used in this study as a reference by which the new biomarker was compared (Kobayashi). Data from previous reports have shown a correlation between AD and DNA methylation levels in the brain of patients living with the disease (Kobayashi). Previously, Kobayashi et al. had discovered a location of increased methylation relating to symptoms of dementia, but were unable to distinguish between AD and amnesic mild cognitive impairment (aMCI)—locating a specific region of DNA that, when methylated, results in Alzheimer's disease, was the goal of this study.

A cohort of 30 patients with AD and 28 patients with aMCI were enrolled in the study, having been previously diagnosed via the U. S. National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (Kobayashi, 2016). Patients were also assessed for AD and aMCI using the mini-mental state exam (MMSE) and frontal assessment battery (FAB) both of which reflect cognitive impairment (Kobayashi). With these clinical diagnostic measures, more quantitative diagnostic tools could be utilized and compared for consistency and efficacy.

From the data collected from the DNA methylation sequencing (Figure F), multiple genes were identified as correlating with MMSE and FAB scores—having P values <0.05 (Kobayashi, 2016). Among these genes, Kobayashi et al. focused on *NCAPH2/LMF2,* as it had the lowest P values—consequently, pyrosequencing was conducted to specifically look at the decreased methylation levels at this gene. The results showed a difference in the methylation levels of *NCAPH2/LMF2* between the normal controls and AD or aMCI, however, the difference in levels between the two neurological diseases was "smaller than between [the controls] and aMCI or AD" suggesting that these two diseases are similar (Kobayashi).

One limitation to this study is the ability to distinguish between aMCI and AD. Because patients with either disease have methylation at *NCAPH2/LMF2* and the level of methylation is similar, discriminating between the two proves to be both difficult, and non-distinct (Kobayashi, 2016). Additionally, aMCI does not necessary progress to AD, so if a patient was tested for methylation at *NCAPH2/LMF2*, a diagnosis could not be defined, and if aMCI was suspected, it may or may not progress to AD—this information is not helpful in diagnosing, treating, or monitoring the disease (Kobayashi).

The theory regarding *NCAPH2/LMF2* and the results of increased methylation at this locus require a lot of additional testing, increased sample size, and further information concerning the association between methylation and the clinical presentation of AD. Questions remain after the study: Does the methylation level at *NCAPH2/LMF2* reflect degeneration of the brain or is this gene one of the causes that results in cognitive dysfunction seen in AD patients clinically? Does this decreased methylation contribute to the accumulation of amyloid beta plaques or tau tangles? Will the methylation levels change with any sort of treatment? And, if so, would the changing methylation levels result in improved clinical presentations?

The idea behind discovering a gene that causes a resultant disease is, obviously, not a new concept, but when the cause of the disease is still uncertain, locating a gene that corresponds to a clinical presentation has proven even more difficult. Kobayashi et al. (2016) utilized the information that has been proven to be correlated to AD (APOE allele 4, amyloid beta, and tau; MMSE and FAB assessments; NINCDS-ADRDA criteria) and attempted to correlate data from DNA, which made suggestions for possible biomarkers, but also left a lot of questions unanswered.

Positron Emission Tomography Tracers

Positron emission tomography (PET) scans have been utilized in the diagnosis of AD by creating various radiotracers targeting known potentially-pathologic proteins in the brain, and determining the presence or extent of the disease. While the magnetic resonance imaging (MRI) imaging modality creates superior "spatial resolution" (Shimojo, 2015), PET scans provide the "greatest in the quantification of the kinetics of neuronal receptor molecules," (Shimojo). There is a proposed model, Braak's model, which uses the *post mortem* pathology samples obtained from patients diagnosed with AD, to categorize the severity of the disease based on quantifiable senile plaques (SPs) and neurofibrillary tangles (NFTs) (Shimojo). Using this model, if SPs and NFTs could be visualized *in vivo* via PET scan, with the use of radiotracers, a diagnosis of AD could be made, staged, and monitored.

In this study, Shimojo et al. (2015) focused their research for PET radiotracers on those targeting Aβ and tau proteins as these are the proteins measured in *post partum* brain tissue sampling, and will determine a diagnosis of AD, and the stage at which the disease has progressed (Figure G). Three radiotracers have already been identified and approved by the FDA for diagnostic application in the clinical setting: [^{18}F]3'-FPiB, [^{18}F]AV-1, and [^{18}F]AV-45 (Shimojo). These tracers are specific to Aβ/SPs detection, but have shown difficulty in differentiating between retention level in the white matter of the brain, and consequently require further work in creating a more distinguishable radiotracer. Although there are not currently any FDA approved tau radiotracers, several studies have been shown to be "promising" results, and warrant further testing (Shimojo).

In addition to Aβ and tau proteins, other "disease-associated pathologies such as aberration of energy metabolism, glial inflammation, dysfunction of calcium homeostasis, and

imbalanced neuronal activity can also be targeted as potential biomarkers of AD progression," (Shimojo, 2015). Several of these targets have been previously discussed as biomarkers in other modalities—such as proteins in CSF (Heslegrave, 2016). The difficulty in using these biomarkers is distinguishing between the various targets with the specific radiotracers, as well as the lack of definitive information surrounding the effect of, for example, neuroinflammation on the progression or clinical presentation of AD.

While the idea of utilizing PET scans in the diagnosis, treatment, and monitoring of AD seems logical, the struggle remains to find a radiotracer that is distinct for AD. Additionally, setting up a facility capable of running PET scans is costly and requires specialized equipment, as well as special precautions due to the use of radiation (Shimojo, 2016). The images are helpful in supporting a diagnosis of AD, but additional diagnostic modalities to complement the PET scans further enhance the support given to the clinical diagnosis—support that a PET scan alone cannot uphold (Shimojo).

Employment of PET scans for AD diagnosis *in vivo* is a research field that has accomplished much so far, and appears to have much opportunity for utilization in the future (Shimojo, 2016). There is room, however, for improvement, as far as creating radiotracers more specific to known AD biomarkers, Aβ and tau (Shimojo). Even further, would be distinguishing tracers specific to toxic tau species versus those that are neuroprotective—allowing for earlier diagnosis of AD (Shimojo).

In a study conducted by Jena et al. (2015), PET scans were combined with MRI imaging to create a more comprehensive image of a brain affected by AD. With MRI imaging, the neuronal loss can be quantitatively measured (Jena). Additionally, the PET scan can be conducted with Aβ radiotracers, highlighting accumulations of amyloid deposits (Jena). With

these two modalities, and a clinical diagnosis, a “higher diagnostic accuracy” is achieved than with a single method (Jena). Other studies have suggested that due to the ability of the MRI to detect brain degeneration, it can be used to diagnose preclinical or mild AD (Jena). Measurements of brain atrophy are limited by the fact that neuronal loss can occur naturally with age regardless of the presence of AD—so it is not necessarily specific to the disease, and is therefore not definitively diagnostic.

Near-infrared Fluorescence Imaging Probes

Tong et al. studied molecular probes utilized in the diagnosis of Alzheimer’s disease by targeting Aβ plaques both *in vivo* and *in vitro.* Ideally these probes could be used to diagnose and monitor the progression of the disease while remaining minimally invasive, cost effective, and readily available (Tong, 2014). While other imaging modalities have a place in the study of AD, none of them can definitively diagnose AD, and can “only support other diagnostic criteria,” (Tong). Additionally, near-infrared fluorescence (NIRF) imaging is comparatively inexpensive, nonradioactive, offers real-time imaging, is widely available, and the images offer high resolution (depending on the technique used), making NIRF an “attractive alternative” to MRI, PET or SPECT imaging (Tong).

While many probes for NIRF imaging are commercially available, some have shown difficulty in crossing the blood-brain barrier (BBB) due to their large molecular size, and consequently have no application in the diagnosis of AD (Tong, 2014). According to Tong et al., there are several criteria that must be met in order for the probes to work in NIRF imaging:

1. Suitable wavelength of excitation and emission – 600-900 nm
2. High BBB permeability – log*P* values 2-3.5

3. High affinity for specific labeling of the Aβ plaques in the brain and low affinity for other proteins
4. Rapid clearance of unbound dye from the brain
5. Significant changes in the probe fluorescence properties upon binding to Aβ plaques [so that these changes can be detected]
6. Molecular weight less than 600 Daltons

Because of all of these requirements, far fewer NIRF probes have been created successfully than PET/SPECT probes (Tong).

In this study, Tong et al. (2014) discussed six different kinds of NIRF probes that target Aβ *in vivo*: NIAD-4, AOI-987, curcumin derivatives, BODIPY based probes, THK-265, and DANIR 2c. For one reason, or another, most of the probes missed one of the criteria, limiting their ability to serve as a diagnostic tool for AD (Tong, 2014). However, the curcumin derivatives were able to reach the targeted proteins, bind and show high affinity specifically for Aβ proteins, and show significant enough changes when bound to be detected (Tong). Tong et al also compared the curcumin probes to well-known Aβ PET probe, PIB, which further supported these findings. THK-265 also showed great specificity and detection of Aβ aggregation, suggesting its use in the monitoring and "evaluating different cerebral Aβ aggregation levels in different stage of AD progression…" (Tong).

Due to the distinct specifications required to make NIRF probes viable for diagnostic tools in AD, there are a limited number currently available, however, this is a field that merits further research. There are several qualities that make NIRF imaging superior to other imaging modalities, but these modalities do not require such strict specifications to function as biomarkers for AD. Fu et al. (2015), conducted a similar experiment but focused on the probe

DANIR 8c. By slightly altering the probe, it was shown to “efficiently distinguish between [mice diagnosed with AD] and normal controls” suggesting that it is a viable probe for *in vivo* detection of Aβ plaques (Fu). Now that several probes have been found to marginally meet the criteria required for an efficacious biomarker, minor alterations allowing them to work more proficiently are in the future for NIRF imaging.

Conclusion

A method for creating a definitive diagnosis of Alzheimer's disease *in vivo* has not been discovered yet, but researchers are getting closer to achieving that goal. While practitioners can make a clinical diagnosis of AD, a quantitative analysis of the progression of the disease and a method by which AD can be distinguished from other neurological diseases is still unavailable. Currently amyloid beta and tau can be measured in cerebrospinal fluid, traced on a PET scan, and detected via SPECT probes *in vivo*, but the results are not distinct to AD. It is unknown at this point what the specific protein or diagnostic tool will be—CSF biomarkers, plasma biomarkers, gene-specific biomarkers, or an imaging modality, that allow the disease to be measured, monitored, and diagnosed.

Once that point in AD research is reached, and clinicians are able to definitively diagnose his or her patients with Alzheimer's disease, a cascade of information, treatment options, and avenues for research will open—and the world of AD will be rapidly changed. At this very moment, people across the world are searching for that one protein, that one gene, that one molecule, that will bring peace to millions living with this "family disease" (DeFina, 2013).

References

DeFina, P. A., Moser, R. S., Glenn, M., Lichtenstein, J. D., & Fellus, J. (2013). Alzheimer's disease clinical and research update for health care practitioners. *Journal of Aging Research, 2013,* article 207178. doi:10.1155/2013/207178

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). Mini-mental state: A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research, 12*(3), 189-198.

Fu, H., Tu, P., Zhao, L., Dai, J., Liu, B., & Cui, M. (2015, December 30). Amyloid-β deposits target efficient near-infrared fluorescent probes: Synthesis, *in Vitro* evaluation, and *in Vivo* imaging. *Analytical Chemistry, 88*(3), 1944-1950. doi:10.1021/acs.analchem.5b04441

Heslegrave, A., Heywood, W., Paterson, R., Magdalinou, N., Svensson, J., Johansson, P., et al. (2016). Increased cerebrospinal fluid soluble TREM2 concentration in Alzheimer's disease. *Molecular Neurodegeneration, 11*(3). doi:10.1186/s13024-016-0071-x

Horinek, D., Stepan-Buksakowska, I., Szabo, N., Erickson, B. J., Toth, E., Sulc, V., . . . Kincses, Z. T. (2016). Difference in white matter microstructure in differential diagnosis of normal pressure hydrocephalus and Alzheimer's disease. *Clinical Neurology and Neurosurgery, 140*, 52-59. doi:10.1016/j.clineuro.2015.11.010

Jena, A., Renjen, P. N., Taneja, S., Gambhir, A., & Negi, P. (2015). Integrated F-fluorodeoxyglucose positron emission tomography magnetic resonance imaging (F-FDG PET/MRI), a multimodality approach for comprehensive evaluation of dementia patients: A pictorial essay. *Indian Journal of Radiology and Imaging, 25*(4), 342-352.

Kobayashi N., Shinagawa S., Nagata T., Shimada K., Shibata N., Ohnuma T., et al. (2016) Development of biomarkers based on DNA methylation in the NCAPH2/LMF2 promoter region for diagnosis of Alzheimer's disease and amnesic mild cognitive impairment. *PLoS ONE, 11*(1): e0146449. doi:10.1371/journal.pone.0146449

Leung, Y. Y., Toledo, J. B., Nefedov, A., Polikar, R., Raghavan, N., Xie, S. X., Schultz, T., et al. (2015). Identifying amyloid pathology-related cerebrospinal fluid biomarkers for Alzheimer's disease in a multicohort study. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring, 1*(2015), 339-348. doi:10.1016/j.dadm.2015.06.008

National Institute on Aging. (n.d.). *Alzheimer's disease genetics fact sheet.* Retrieved from https://www.nia.nih.gov/alzheimers/publication/alzheimers-disease-genetics-fact-sheet

Nesteruk, M., Nesteruk, T., Styczynska, M., Barczak, A., Mandecka, M., Walecki, J., & Barcikowska-Kotowicz, M. (2015). Predicting the conversion of mild cognitive impairment to Alzheimer's disease based on the volumetric measurements of the selected brain structures in magnetic resonance imaging. *Polish Journal of Neurology and Neurosurgery, 49*, 349-353. doi:10.1016/j.pjnns.2015.09.003

Ortiz, A., Munilla, J., Alvarez-Illan, I., Gorriz, J. M., & Ramirez, J. (2015). Exploratory graphical models of functional and structural connectivity patterns for Alzheimer's disease diagnosis. *Frontiers in Computational Neuroscience, 9*(132), 1-18. doi:10.3389/fncom.2015.00132

Rutten, B. P. (2013, October 22). Current concepts in Alzheimer's disease: Molecules, models and translational perspectives. *Molecular Neurodegeneration, 8,* article 33.

Shimojo, M., Higuchi, M., Suhara, T., & Sahara, N. (2015, December 22). Imaging multimodalities for dissecting Alzheimer's disease: Advanced technologies of positron

emission tomography and fluorescence imaging. *Frontiers in Neuroscience, 9*(482). doi:10.3389/fnins.2015.00482

Sorensen, L., Igel, C., Hansen, N. L., Osler, M., Lauritzen, M., Rostrup, E., & Nielsen, M. (2016). Early detection of Alzheimer's disease using MRI hippocampal texture. *Human Brain Mapping, 37*(3), 1148-1161. doi:10.1002/hbm.23091

Tong, H., Lou, K., & Wang, W. (2015). Near-infrared fluorescent probes for imaging of amyloid plaques in Alzheimer's disease. *Acta Pharmaceutica Sinica B, 5*(1), 25-33. doi:10.1016/j.apsb.2014.12.006

Werner, P., Karnieli-Miller, O., & Eidelman, S. (2013). Current knowledge and future directions about the disclosure of dementia: A systematic review of the first decade of the 21st century. *Alzheimer's & Dementia, 9*(2013), E74-E88. doi:10.1016/j.jalz.2012.02.006

Yu, S., Liu, Y., Liu, Y., Jiao, S., Liu, L., Wang, Y., & Fu, W. (2015). Diagnostic utility of VEGF and soluble CD40L levels in serum of Alzheimer's patients. *Clinica Chimica Acta, 453*, 154-159. doi:10.1016/j.cca.2015.12.018

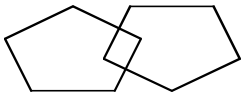
Figures

Figure A: Mini-Mental State Exam (Folstein, 1975)

Mini-Mental State Examination (MMSE)

Patient's Name: _____ Date: _____

Instructions: Score one point for each correct response within each question or activity.

Maximum Score	Patient's Score	Questions
5		"What is the year? Season? Date? Day? Month?"
5		"Where are we now? State? County? Town/city? Hospital? Floor?"
3		The examiner names three unrelated objects clearly and slowly, then the instructor asks the patient to name all three of them. The patient's response is used for scoring. The examiner repeats them until patient learns all of them, if possible.
5		"I would like you to count backward from 100 by sevens." (93, 86, 79, 72, 65, …) Alternative: "Spell WORLD backwards." (D-L-R-O-W)
3		"Earlier I told you the names of three things. Can you tell me what those were?"
2		Show the patient two simple objects, such as a wristwatch and a pencil, and ask the patient to name them.
1		"Repeat the phrase: 'No ifs, ands, or buts.'"
3		"Take the paper in your right hand, fold it in half, and put it on the floor." (The examiner gives the patient a piece of blank paper.)
1		"Please read this and do what it says." (Written instruction is "Close your eyes.")
1		"Make up and write a sentence about anything." (This sentence must contain a noun and a verb.)
1		"Please copy this picture." (The examiner gives the patient a blank piece of paper and asks him/her to draw the symbol below. All 10 angles must be present and two must intersect.) 
30		TOTAL

http://www.dementiatoday.com/wp-content/uploads/2012/06/MiniMentalStateExamination.pdf

Figure B: Demographic data, clinical characteristics and CSF levels of neurogranin, and YKL-40 (Janelidze, 2015)

	Control (n = 53)	sMCI (n = 62)	MCI−AD (n = 35)	AD (n = 74)	DLB/PDD (n = 47)	VaD (n = 34)	FTD (n = 33)
Age	75.3 (6.4)	69.2 (7.5)[a]	75.0 (7.6)[b]	76.4 (7.4)[b]	74.5 (6.3)[b]	75.7 (7.8)[b]	71.7 (6.7)[c, d, e]
Sex (% female)	70%	56%	66%	68%	40%[d, f, g]	47%[c, h]	51%
MMSE	28.6 (1.8)	28.2 (1.2)	26.4 (1.7)[a, b]	19.4 (3.3)[a, b, i]	21.9 (5.1)[a, b, d, i]	21.5 (4.4)[a, b, d, i]	21.8 (6.6)[a, b, i]
APOE 1 or 2 ε4 alleles	31%	53%	80%[a, j]	65%[a, k]	54%[c, g]	24%[i, k, l, m]	27%[l]
Neurogranin, pg/mL	557 (328)	542 (279)	652 (348)	711 (404)[c]	480 (312)[d, g]	313 (150)[a, b, i, l, m]	370 (194)[f, i, j, l]
YKL−40, ng/mL	200 (64)	184 (69)	219 (59)	248 (70)[a, b]	217 (65)[h]	221 (69)	222 (59)[f, j, n]
Aβ42, pg/mL	668 (287)	486 (200)[a]	314 (79)[a, b]	260 (106)[a, b]	340 (173)[a, b, h]	397 (187)[a, d, k]	676 (289)[b, i, l, o, q]
Aβ40, pg/mL	5136 (1531)	3821 (1377)[a]	4219 (1327)[f]	3892 (1383)[a]	3170 (1137)[a, h, j, r]	3209 (1277)[a, h, k, r]	4470 (1550)[k, h, o, q]
Tau, pg/mL	467 (191)	437 (175)	643 (224)[a, b]	768 (267)[a, b, r]	472 (171)[j, l]	436 (191)[j, l]	382 (205)[j, l]
Aβ42/neurogranin	1.57 (0.99)	1.10 (0.60)[f]	0.65 (0.46)[a, k]	0.50 (0.40)[a, b]	0.91 (0.55)[a, h]	1.62 (1.20)[j, i, l, o]	2.40 (2.03)[a, b, i, l, o]
Aβ42/YKL−40	3.82 (2.23)	2.83 (1.18)[a]	1.57 (0.76)[a, b]	1.10 (0.47)[a, b]	1.69 (0.90)[a, b, h]	1.93 (0.95)[a, d, k]	3.08 (1.24)[f, i, l, o, p]
Aβ42/Aβ40	0.13 (0.04)	0.13 (0.04)	0.08 (0.02)[a, b]	0.07 (0.02)[a, b]	0.11 (0.04)[a, i, k, l]	0.13 (0.04)[i, l]	0.15 (0.04)[e, i, j, l, o]
Aβ42/tau	1.66 (0.82)	1.25 (0.56)[c]	0.54 (0.26)[a, k]	0.38 (0.23)[a, b]	0.82 (0.50)[a]	1.02 (0.54)[c, h]	2.57 (3.45)[b, f, i, l, o, q]

Figure C: Characteristics of AD patients and controls (Heslegrave, 2016)

Subject details	Controls (n = 22)	AD patients (n = 37)	p value
Gender (F/M), no (%)	10(45)/12(55)	19(53)/18(47)	0.6
Age, years (mean ± SD)	69.2 ± 8.0	70.51 ± 7.5	0.54
MMSE score (median IQR)	29.00(26.50-29.00)	22.00(18.00-25.00	<0.0001
APOE ε4 positive (%)	33 %	67 %	0.0002
Plasma CRP (mg/L), median (IQR)	2.095 (0.8425 – 4.7535) n = 16	1.020 (0.6500 – 1.295) n = 25	0.0543
CSF Biomarkers			
Aβ1-42 (pg/ml), median (IQR)	978.5(821.8-1045)	367.0(301.0-433.0)	<0.0001
T-tau (pg/ml), median (IQR)	239.5(196.3-279.0)	596.0(455.0-858.5)	<0.0001
P-tau$_{181}$ (pg/ml), median (IQR)	45.0(36.8-58.3)	95.0(75.0-111.5)	<0.0001
T-tau/Aβ1-42 ratio, median (IQR)	0.25(0.19-0.33)	1.56(1.24-2.55)	<0.0001
sTREM2 (pg/ml), median (IQR)	195.6(131.0-240.7)	231.2(172.5-305.4)	0.0457

Data expressed as mean ± SD or median (IQR) as appropriate. Probability values (p) denote differences between control and AD. A χ^2 test was used for gender and *APOE* genotype comparisons. CSF biomarkers and sTREM2 were evaluated using the Mann-Whitney U test

Figure D: Blood-Based Biomarker Results from Yu et al.

	Subjects with AD	Control subjects
Concentration of VEGF	93.15 pg/ml	35.04 pg/ml
Concentration of sCD40L	9222.00 pg/ml	1854.00 pg/ml
Serum Lg(CD40L)	3.91±0.53	2.82±1.01
Serum Lg(VEGF)	1.95±0.50	1.10±1.06

Figure E: Diagnostic accuracy of Lg(VEGF), Lg(sCD40L) and a combination of the two biomarkers to distinguish the AD cases from control participants. (Yu, 2016)

	AUC (95% Cl)	Optimal threshold	Sensitivity (95% Cl)
Lg(VEGF)	0.731 (0.624-0.839)	1.595	0.78 (0.64-0.88)
Lg(sCD40L)	0.824 (0.737-0.910)	3.600	0.76 (0.62-0.87)
Lg(VEGF) + Lg(sCD40L)	0.858 (0.775-0.941)	0.765	0.80 (0.66-0.90)

Figure F: Spearman's Rank Correlations between Methylation Levels and MMSE, or FAB Scores (Kobayashi, 2016)

Target ID	GENE NAME	MMSE			FAB		
		ρ	*P*	n	*ρ*	*P*	n
cg01756799	*COASY*	-0.76	0.031 *	8	-0.88	0.021 *	6
cg06695761	*SGCE/PEG10*	-0.66	0.076	8	-0.70	0.123	6
cg08727202	*MPST/TST*	-0.65	0.083	8	-0.76	0.080	6
cg09898695	*SPINT1*	-0.73	0.040 *	8	-0.40	0.439	6
cg13523072	*COASY*	-0.49	0.217	8	-0.82	0.046 *	6
cg13947830	*MIB2*	-0.64	0.091	8	-0.40	0.439	6
cg19205533	*RERG*	-0.90	0.002 **	8	-0.52	0.295	6
cg23779106	*DUSP12*	-0.42	0.301	8	0.40	0.439	6
cg25152348	*NCAPH2/LMF2*	-0.89	0.003 **	8	-0.94	0.005 **	6
cg26812418	*CPE*	-0.68	0.062	8	-0.15	0.774	6
cg27173717	*MFSD2A*	-0.64	0.091	8	-0.40	0.439	6

* $P < 0.05$

** $P < 0.01$

Figure G: Reference list for Aβ and Tau PET radiotracers (Shimojo, 2015)

Target	Radiotracer for PET imaging of SPs or NFTs	Approval for clinical use	References
Aβ/SPs	[^{18}F]FDDNP		Barrio et al., 1999; Shoghi-Jadid et al., 2002; Small et al., 2006; Thompson et al., 2009; Smid et al., 2013
Aβ/SPs	[^{11}C]PiB		Klunk et al., 2004
Aβ/SPs	[^{18}F]3′-FPiB	Flutemetamol; Vizamyl, GE healthcare	Yang et al., 2012
Aβ/SPs	[^{18}F]AV-1	Florbetaben; Neuraceq, Piramal imaging	Rowe et al., 2008
Aβ/SPs	[^{18}F]AV-45	Florbetapir; Amyvid, Eli Lily	Choi et al., 2009
Tau/NFTs	[^{18}F]THK523		Fodero-Tavoletti et al., 2011; Harada et al., 2013
Tau/NFTs	[^{18}F]THK5105		Okamura et al., 2014
Tau/NFTs	[^{18}F]THK5117		Okamura et al., 2014
Tau/NFTs	[^{18}F]THK5351		Villemagne et al., 2014
Tau/NFTs	[^{18}F]T807		Xia et al., 2013
Tau/NFTs	[^{18}F]T808		Zhang et al., 2012
Tau/NFTs	[^{11}C]PBB3		Maruyama et al., 2013

Appendix of Terms and Abbreviations

Alzheimer's disease (AD)

Amyloid Beta (Aβ)

Amyotrophic Lateral Sclerosis (ALS)

Apolipoprotein E (APOE): Gene associated with the development of symptoms of AD. Different alleles have been shown to correlate to different presentations of AD.

- APOE ε2 – rare allele that has been suggested to delay the onset of AD to later-in-life; thought to be a protectant *against* AD
- APOE ε3 – neutral risk associated with AD—neither seems to slow or advance the progression of the disease
- APOE ε4 – associated with increased risk of AD and earlier onset of the symptoms. Patients can have 0, 1, or 2 APOE ε4 alleles, and the more alleles present, the more increased the risk of developing the disease

Blood-Brain Barrier (BBB)

Cerebrospinal Fluid (CSF)

Frontotemporal Degeneration (FTD)

Magnetic Resonance Imaging (MRI)

Mild Cognitive Impairment (MCI)

Mini Mental State Exam (MMSE)

Neurofibrillary Tangles (NFTs)

Near-Infrared Fluorescence (NIRF)

Parkinson's disease (PD)

Positron Emission Tomography (PET)

Single Photon Emission Computed Tomography (SPECT)

Vascular Endothelial Growth Factor (VEGF)

Abstract

Alzheimer's disease (AD) is an incurable, poorly treatable disease that currently afflicts approximately 5 million people America. Presently AD is only definitively diagnosed after *post-mortem* examination of the brain for the presence of characteristic histopathological features, predominant amongst which are amyloid beta (Aβ) protein-containing plaques and neurofibrillary tangles (NFTs) containing hyperphosphorylated protein tau. Nonetheless, there is a pressing need to diagnose the presence, and quantify severity of AD in the living patient in order to support family planning; assign patients to experimental drug trials early enough that an effect might be seen; and to allow clinicians and researchers to follow patient response to experimental treatments. To achieve these needs, new biomarkers are being identified and tested for their applicability in the clinical monitoring of AD. A biomarker is any measurable substance in an organism that reliably correlates with disease status and severity. Current biomarkers can support a prior achieved diagnosis via mental status examinations and clinical findings, but do not efficiently distinguish AD from other neurological diseases or accurately quantify disease severity. Most biomarker research to date has focused on disease-related proteins found in blood or cerebrospinal fluid (CSF). In more recent approaches, the methylation of specific coding regions on DNA have been correlated with AD patients' symptoms, but thus far these studies have not been adequately powered to demonstrate the biomarker's utility as a diagnostic tool. *In vivo* imaging, particularly using radiological tracers in conjunction with positron emission tomography (PET), offers the ability to measure AD-associated brain proteins such as Aβ and tau in the living patient but do not completely distinguish between normal and "toxic" forms of these proteins. Overcoming these limitations should facilitate faster, less expensive and more powerful clinical trials and support development of new therapies to actually slow AD dementia.