Is Detection of Preclinical Alzheimer's Disease Possible?

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Abstract

Alzheimer's disease, the most common form of dementia, is a neurodegenerative disease that causes progressive memory loss and severe cognitive impairment with time. As the sixth leading cause of death in the United States, Alzheimer's disease kills more people than breast cancer and prostate cancer combined, and there are over five million Americans suffering from Alzheimer's disease today. A diagnosis of probable Alzheimer's disease is typically given after rigorous neurological and psychological examination, neuroimaging, and cognitive testing, although the diagnosis cannot be confirmed unless a postmortem examination finds specific pathological lesions in the brain. The lack of proper diagnostic technique often leads to patients being diagnosed after years of the disease's progression, at which point there is already significant neurological damage. Biomarkers for Alzheimer's disease are therefore crucial to accurately monitor the disease's progression and eventually determine the effectiveness of certain therapies. There are countless studies being performed to discover the different biomarker possibilities for detecting Alzheimer's disease in its preclinical stage. Constructed on multiple original scientific research articles, this paper focuses on two such approaches toward preclinical Alzheimer's disease detection, specifically focusing on blood-based biomarkers. These detection methods have been developed based on the two pathological characteristics of the disease, beta-amyloid plaques and neurofibrillary tangles in the brain that lead to cerebral atrophy. The ADAM10 protease can be used as an Alzheimer's disease biomarker by having reduced levels in patients with the disease, lending to the hypothesis that its reduction leads to the formation of beta-amyloid plaques. Using plasma-based tests to measure inflammatory-related responses such as cytokines and plasma signal proteins to neurofibrillary tangles is another effective way in detecting preclinical Alzheimer's disease. These biomarker possibilities show promising opportunities to detect Alzheimer's disease before or as soon as symptoms appear, thus enabling patients to receive optimal care and treatment.

Introduction

According to the 2011 Criteria and Guidelines for Alzheimer's disease developed by the National Institute of Health and the Alzheimer's Association, there are three stages of Alzheimer's disease. In the first stage, preclinical Alzheimer's disease, patients exhibit no symptoms of memory loss or changes in behavior, but begin to form progressive pathological characteristics of the disease in the brain, cerebrospinal fluid, and blood. The second stage, mild cognitive impairment, or MCI, due to Alzheimer's disease indicates noticeable changes in behavior and cognition that do not affect everyday life function and activities. The final stage is dementia due to Alzheimer's disease. This is the stage in which a person exhibits distinct memory loss and cognitive decline that severely impairs everyday function (www.alz.org).

Although there are already measurable pathological changes in the brain, cerebrospinal fluid, and blood during the preclinical stage of Alzheimer's disease, the changes are rarely detected or tested for due to the lack of symptoms. While preclinical Alzheimer's is considered the first stage of the disease, the 2011 criteria and guidelines for Alzheimer's disease do not list diagnostic criteria that can be used in diagnosing it, causing the typical Alzheimer's disease patient to be first diagnosed after years of the disease's progression, and only after symptoms appear.

In the past two decades, there has been a huge effort to develop disease-modifying drugs to slow or stop the progression of Alzheimer's disease. These treatments are predicted to be most effective in the preclinical and early mild cognitive impairment stages. However, because most patients partaking in clinical trials are already in advanced stages of the disease, many of these trials have failed. Therefore, specific, easily-identifiable and accessible biomarkers are crucial for diagnosing patients in the preclinical stage so that their fitness for disease-modifying treatments can be determined, and the effectiveness of such treatments can be accurately monitored (www.alz.org). Understanding specific biomarkers would also lend patients more time to plan for their future and would give them opportunity to be involved in decisions involving their home care and legal matters while their cognition is still intact (Gomez Ravetti, Moscato, 2008).

Current detection mechanisms of Alzheimer's disease include brain imaging and cerebrospinal fluid testing. These methods, however, are expensive and invasive, and are not easily accessible to large portions of the population (O'Bryant et al., 2011). Additionally, these methods are inconclusive and are usually only performed once the disease has reached the mild cognitive impairment or dementia stage and has already progressed significantly.

Researchers are therefore in search of conclusive biomarkers that can be used in routine screenings and detection of preclinical Alzheimer's disease (Berg, 2008). Peripheral blood-based biomarkers are ideal for Alzheimer's disease screening, as they can be easily accessed via routine venipuncture, which is cost-effective, noninvasive, and available to majority of the population (O'Bryant et al., 2011). This paper explores several blood-based biomarker possibilities that can be used as routine screenings for preclinical or early-onset Alzheimer's disease.

Alzheimer's Disease Pathology

Although the exact pathological cause of Alzheimer's disease is still unknown, there are two major pathological characteristics that researchers have pinpointed to indicate the disease and its progression. These characteristics are excessive beta-amyloid peptide plaques and phosphorylated tau protein deposition in

the brain. Through different mechanisms, these characteristics both cause neuronal death and eventual atrophy that spreads throughout the brain (Manzine et al., 2013).

In a normal, nonamyloidogenic pathway, amyloid precursor protein, or APP, an enzyme embedded in the cell membrane of neurons, is cleaved by alpha-secretase and releases a fragment called aAPP. aAPP is a large and insoluble molecule, and is believed to have a protective effect on the neural cells it occupies. aAPPs protect the neurons of the hippocampus against excito-toxicity, A beta toxicity, and glucose deprivation (Furukawa et al., 1996), and enhance learning and memory function. In patients with Alzheimer's disease, there is a decrease in APP processing via a-secretases (Manzine et. al., 2013). Instead, APP is cleaved by beta-secretase, followed by gamma-secretase cleavage. Beta-amyloid, the fragment produced by this pathway, is shorter than aAPP, and when beta-amyloid fragments aggregate, they become insoluble, forming beta-amyloid plaques. These plaques interfere with inter-neuronal communication, causing information transfer at synapses to fail, eventually leading to neuronal death (Cummings et al., 1996).

The second pathological characteristic of Alzheimer's disease is neurofibrillary tangles caused by abnormal tau protein deposition in the brain. In normal neurons, the tau protein attaches to microtubules and stabilizes the important internal structures of the cell by acting as a firm, scaffolding-like structure. Nutrients are carried throughout this structure, and the tau protein is essential for normal cell function and nutrient transport. In patients with Alzheimer's disease, however, the tau protein is modified and behaves differently. Tau protein separates from the microtubules in neurons of patients with Alzheimer's disease, causing the structure to fall apart. Strands of tau combine and form tangles inside the neuron, disabling the transport system and eventually destroying the cell (Ingelsson et al., 2004).

The accumulation of both beta amyloid plaque and neurofibrillary tangles leads to cell death. In patients with Alzheimer's disease, the abundance of cell death causes the brain to shrink in size and many important functions, such as memory, to fail. Atrophy begins in the lateral entorhinal cortex, which together with the hippocampus plays an integral role in long-term memory storage. Over time, the brain atrophy spreads to other areas of the cerebral cortex, specifically to the parietal cortex, the region of the brain associated with navigation and spatial orientation (Liu et al., 2013), leading to severe cognitive decline.

Researchers are still unsure of the direct causes of beta-amyloid plaque accumulation and neurofibrillary tangle formation. They have, however, pinpointed these characteristics as the cause of cerebral atrophy and cognitive decline in patients with Alzheimer's disease. It is therefore useful to utilize these known pathological markers of Alzheimer's disease as the basis for biomarker research, as they are present not only in the mild cognitive impairment and severe Alzheimer's disease stages, but begin to form in the preclinical stage, prior to the onset of cognitive symptoms (www.alz.org).

Methods

Research was done by studying original research articles and scientific papers found on the Touro College online library. Specific scientific databases such as Proquest and EBSCO were perused, and additional information was obtained by analyzing articles found.

Discussion:
Current Detection of Alzheimer's Disease Progression

Today, when there is concern for possible Alzheimer's disease, there is no definitive step to reach a diagnosis. Instead, a series of steps must be taken to ultimately lead to a probable diagnosis of the disease. Even then, it is only when neurofibrillary tangles
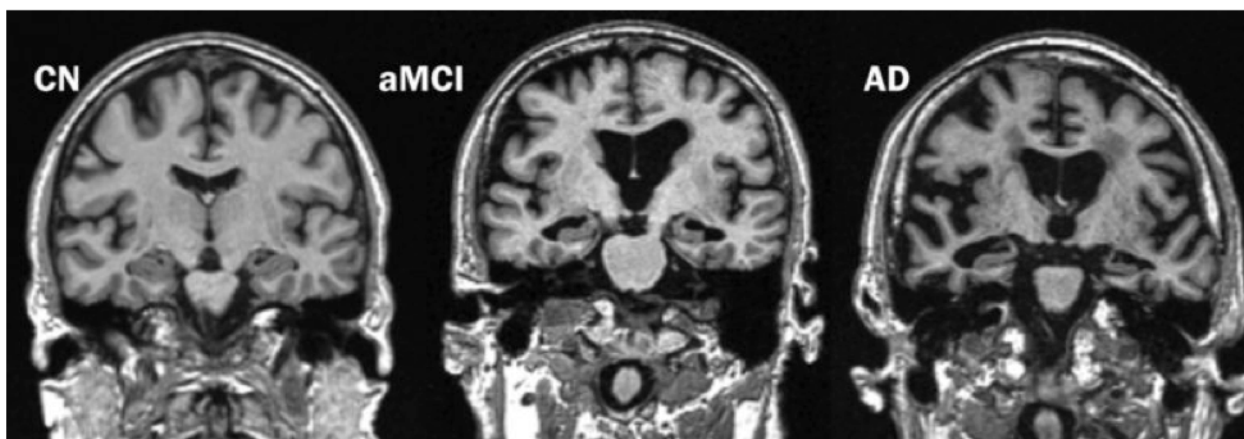

Figure 1: MRI scans of the brains of (i) cognitively normal (CN) patient, (ii) patient with amnesic mild cognitive impairment, and (iii) patient with Alzheimer's disease (Vemuri, Jack, 2010).

(NFTs) in neurons, amyloid plaques, and synapse or neuronal loss and atrophy are found upon postmortem examination of the brain that Alzheimer's disease can be fully confirmed (Berti et al., 2010).

To reach a probable diagnosis of Alzheimer's Disease, a mini mental state exam, or MMSE, is first performed, in which the patient is asked to fulfill certain tasks and answer a variety of questions ranging from recalling the date and time to repeating words or sentences and copying drawings and shapes. A gross score is assigned, and a score ranking of "cognitively impaired" will cause a doctor to send a patient for further testing (O'Bryant et al., 2008).

Blood tests will be done to rule out other causes of dementia, such as abnormal thyroid function or vitamin B-12 levels (Agarwal et al., 2008). The patient may then be referred to a neurologist or sent for brain imaging to detect more specific Alzheimer's disease pathological markers or other causes of cognitive impairment by looking for blood accumulation, brain tumors, strokes, and change in brain size.

While brain imaging such as magnetic resonance imaging (MRI) and positron emission topography (PET) scans are currently used on patients in the mild cognitive impairment or late Alzheimer's dementia stage, there is still much uncertainty regarding the use of such imaging techniques in detecting the disease in its preclinical stage. As seen in figure 1, MRI imaging shows clear cerebral atrophy in patients with mild cognitive impairment and Alzheimer's disease when compared to patients with normal cognition (Vemuri, Jack, 2010). Several studies have shown that MRI detection of atrophy of the medial temporal lobes (MTL), the brain region responsible for language and long-term memory, can be an indication of early AD and predicts conversion to dementia (Rusinek et. al., 2003). Additionally, Fluoro-2-deoxy-D-glucose (FDG) PET scanning has played the leading neuroimaging role in the early detection of Alzheimer's disease by using estimates of cerebral metabolic rate of glucose (CMRglc), an indication of synaptic density and function. Patients with Alzheimer's disease will exhibit CMRglc reductions that are visible on FDG-PET scans, which can even be detected before the onset of Alzheimer's symptoms (Berti et al., 2010). However, FDG-PET and MRI scans are limited as they lack disease specificity; other dementias exhibit reductions in CMRglc, as well, and there can be other causes of brain atrophy detected on an MRI (Wollman, Prohovnik, 2003).

Currently, cerebrospinal fluid (CSF) levels of tau protein and beta-amyloid plaques are the most reliable and widespread protein biomarkers used for Alzheimer's disease detection. This is because cerebrospinal fluid interacts directly with extracellular brain space and can therefore be used as an accurate marker for pathological changes in the brain (Hampel et al., 2012). However, obtaining cerebrospinal fluid requires a lumbar puncture, or "spinal tap", a procedure that is expensive, painful, and time-consuming, and usually needs to be performed in a hospital setting. Testing CSF is therefore not a practical way to screen for preclinical Alzheimer's disease.

Genetic Risk Factors of Alzheimer's Disease

Although environmental factors such as diet, exercise, and education play a role in the development of Alzheimer's disease, twin studies strongly suggest that genetics is the major factor in its development (Gatz et. al., 2006). While many genes have been suggested as genetic risk factors, only one, the ε4 allele of the apolipoprotein E, or APOE, gene has been confirmed to directly correlate to Alzheimer's disease development (Corder et. al., 1993).

APOE, a glycoprotein composed of 299 amino acids, functions as a ligand, combining with lipids to form lipoproteins. APOE is essential for cholesterol homeostasis throughout the body, and is the principal cholesterol carrier in the brain (Puglielli et al. 2003).

Every person inherits one copy of the APOE gene from each parent. The APOE gene has three common single nucleotide polymorphisms (SNPs) leading to three common isoforms of APOE: Apoε2, Apoε3, and Apoε4. The most common form of APOE is ε3, with about 60 percent of the U.S. population inheriting one ε3 gene from each parent. Only about 20 to 30 percent of the U.S. population carries one or two copies of the ε4 gene, and 10 to 20 percent of the population has one or two copies of ε2. Despite these alleles differing in only one or two amino acids, they have drastically different functions and cause stark differences in the genotype (Raber et al. 2004).

Numerous studies have established that the Apoε3 form neither increases nor decreases the risk of developing Alzheimer's disease, and that the Apoε2 form may decrease the risk of developing the disease. The Apoε4 allele, however, is a strong genetic risk factor for the development of Alzheimer's disease (Corder et al., 1993). Compared to people who possess no ε4 allele, people who are heterozygous for the ε4 allele exhibit a 2-3-fold increase in developing Alzheimer's disease, and people who are homozygous for the ε4 allele exhibit a 12-fold increase in developing the disease (Bertram et al., 2009).

Yet despite the strong correlation between patients with the Apoε4 allele and the development of Alzheimer's disease, the mechanism of how Apoε4 causes an increase in AB-deposition is still not fully understood (Holtzman et al., 2012). Furthermore, inheriting the ε4 allele does not guarantee that someone will develop the disease. The ε4 allele had a significantly weaker effect in some ethnic groups, such as African Americans and Hispanics (Farrer et al., 1997), suggesting that there are other environmental and genetic factors involved in the onset of Alzheimer's disease, and that the predisposition to developing Alzheimer's disease cannot be determined based on this gene alone.

Inflammatory Responses Associated with Alzheimer's Disease

As with any other disease, upon sensing abnormal neuronal degeneration caused by Alzheimer's disease, the body launches an immune response (Cagnin et al., 2001). When neurofibrillary tangles begin to form from abnormal tau proteins, astrocytes and microglia, usually inactive in brain tissue, are activated as part of the initial response (Leung et al., 2013). When activated, microglia become phagocytes and secrete many inflammatory molecules such as cytokines and chemokines, growth factors, complement molecules and adhesion molecules.

Several inflammatory agents have been specifically associated with amyloid deposits, including IL-1, IL-6, a1-antichymotrypsin (ACT), tumor necrosis factor-a (TNF-a), and transforming growth factor (TGF-b) (Mcgeer et al., 2006). When tested in blood plasma, these inflammatory agents showed a positive correlation with beta-amyloid peptide found in cerebrospinal fluid (Sun et al., 2003).

Another study was done comparing cytokine expression in blood plasma to neuropsychological testing and neuroimaging indicating Alzheimer's disease progression. Twenty-seven cytokines and chemokines (chemical messengers that cells release as an immune response) were tested. The study found that five cytokines and chemokines, IL-6, TNF-a, IL- 1ra, IL-10, and IL-13, were inversely related to ventricular volume, whole brain volume, or entorhinal cortex volume in Alzheimer's disease. That is, the greater the immune response to the disease, the smaller the brain volume, or the further along the disease progression. There was also an increase in the level of two cytokines, IL-10 and IFN- γ, when tracked in patients between visits (Leung et. al., 2013).

Aside from acting as an immune response to the atrophy of brain cells, the inflammatory response itself may also be contributing to brain atrophy. According to the amyloid cascade-inflammatory hypothesis, researchers postulate that Alzheimer's disease is caused by the inflammatory response to beta-amyloid deposits and is later intensified by tau protein tangles. As the disease progresses, so does the inflammatory response, and it may be for this reason that multiple attempts at developing disease-modifying drugs have failed, as they fail to take the inflammatory response into account. In this strain, possible therapies dealing with anti-inflammatory agents may show promise for developing successful disease-modifying drugs (McGeer et al., 2013).

In a highly-publicized study, it was hypothesized that the pathological processes related to Alzheimer's disease in the body would trigger an immune response with specific changes in certain signal proteins in the blood. A longitudinal study was done in which patients were tested at the initial diagnosis of Alzheimer's disease with mild cognitive impairment, and were then followed up with two to six years later. Using an algorithm called PAM (partition around medoids), eighteen of the one hundred and twenty proteins tested were identified as Alzheimer's disease predictors,

with a 95% positive agreement and 83% negative agreement with the clinical diagnosis (Ray et al., 2007).

These results showed promising statistics that warranted additional study. The PAM algorithm was then used to predict Alzheimer's disease progression in twenty-two patients with mild cognitive impairment. The prediction of twenty of the twenty-two patients to develop Alzheimer's disease was proven correct when the patients were followed up with two to five years later (Ray et. al., 2007). This highly specific plasma biomarker test can therefore be used to predict Alzheimer's disease years before a clinical diagnosis would typically be made or even before any symptoms appear.

A further study found that of the original nineteen predictor proteins found by Ray et al., just five proteins could be used with 96% accuracy to detect preclinical Alzheimer's disease. Using less proteins caused less errors in prediction, and higher biomarker specificity and accuracy (Gomez Ravetti, Moscato, 2008).

Immune responses to beta-amyloid deposition and neurofibrillary tangles, including activation of microglia and astrocytes to release cytokines and chemokines, can be used as an index of Alzheimer's disease progression (Leung et. al., 2013). Additionally, the use of specific signal proteins can be used to accurately predict development of Alzheimer's disease (Ray et. al., 2007). Treatments aimed at dealing with these inflammatory responses may also prove to be effective although more research is needed to prove that hypothesis (McGeer, McGeer, 2013).

ADAM10 as a Biomarker

Efforts have been made to find biomarkers relating to the pathogenic mechanism of abnormal beta-amyloid aggregation causing the formation of amyloid plaques. The products of the ectodomain shedding process, in which amyloid precursor protein (APP) is cleaved by a-secretase into its soluble counterparts, can be used as detection mechanisms of preclinical Alzheimer's disease by methods similar to those used in measuring the immune response (Pruessmeyer, Ludwig, 2009).

In the amyloidogenic pathway, after APP is cleaved by β-secretase, also called β-site APP-cleaving enzyme 1 (BACE1), it is then cleaved by a γ-secretase, leading to the release of the AB fragment. BACE1 competes with the a-secretase for cleavage of the APP substrate, so that an increase in BACE1 activity causes a decrease in a-secretase activity, and vice versa (Vassar, 2013).

The identity of the a-secretase acting in the amyloid precursor protein cleavage process has never been confirmed. However, in three studies done, three members of the ADAM (A Disintegin And Metalloproteinase) family, ADAM9, ADAM10, and ADAM17, were suggested as possible a-secretases as their overexpression in cells or mice led to an increase in APP cleavage (Koike et al, 1999; Lammich et al, 1999; Slack et al, 2001). To further pinpoint a single protease as the predominant a-secretase, knockout methods were used on the three ADAM metalloproteases.

Only ADAM 10 was found to completely suppress a-secretase cleavage of APP when knocked out. ADAM10, therefore, can be used as a measure of a-secretase cleavage and its reduction can be used as a biomarker to detect preclinical Alzheimer's disease (Kuhn et al., 2013).

In patients with Alzheimer's disease, platelet levels of ADAM10 were reduced. A study was done to determine whether ADAM10 could act as an a-secretase and whether obtaining measurements of ADAM10 from platelets in blood plasma was an accurate measurement of ADAM10 levels in the brain. When compared to levels in cerebral spinal fluid, ADAM10 levels in platelets were similar. This was done because platelets present the highest levels of APP among the peripheral tissues (Colciaghi et al., 2002).

A study was done of two groups of Brazilian elderly people to compare ADAM10 protein levels in participants with Alzheimer's disease versus non-Alzheimer's disease participants. By studying the platelet levels of ADAM10 as the only statistically significant factor to increase probability of Alzheimer's disease, it was found that ADAM10 levels were significantly reduced in patients with Alzheimer's disease versus patients without it. The ADAM10 levels were also stage dependent; the further the disease had progressed, the lower the ADAM10 levels were, indicating a decrease in a-secretase cleavage of APP (Manzine et. al., 2013).

Conclusion

While many ethical concerns can be raised regarding the detrimental psychological effect of a preclinical Alzheimer's disease diagnosis, most patients do not react with a negative emotional response. On the contrary, an Alzheimer's diagnosis often comes as a relief to patients and their families by delivering a cause for a patient's cognitive decline (Carpenter et al., 2008).

Through the implementation of blood-based biomarkers, there is promise for easy detection of preclinical Alzheimer's disease. A combination of testing levels of a-secretases like ADAM10 and specific cytokine and chemokine inflammatory markers can help lead to a definitive preclinical Alzheimer's disease diagnosis. By using a combination of the above testing methods, Alzheimer's disease will be able to be definitively diagnosed before symptoms appear, giving patients time to discuss therapies and make certain decisions on their own. However, these testing methods must still be regarded with caution as many of them have not been tested on a vast enough population to be considered fool-proof. With further and more widespread research on the above-mentioned biomarker possibilities, there is promise for early and even preclinical detection of Alzheimer's disease. Easy and early detection will not only help patients struggling with unknown causes of cognitive decline, but will lend to more reliable research for possible drugs to help cure this prevalent disease.

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