Commentary

Can non-invasive biomarkers lead to an earlier diagnosis of Alzheimer's Disease?

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A lack of consensus on the diagnostic criteria that should be used in the clinical setting limits the early diagnosis of Alzheimer's disease (AD) and increases the backlog of undiagnosed cases. While AD has no cure, the current aim is to diagnose the disease at the pre-symptomatic stage - the key to successful intervention, better understanding, and enabling the admission of improved therapeutic and non-pharmacological interventions. The major issues include the number of people affected, ignorance of the early signs and symptoms by patients, their families and healthcare staff, all of which are exacerbated by the vagueness and variety of symptoms.

There has been a substantial volume of research and development on the new diagnostic algorithms within the last decades with criteria that shift towards the use of biomarkers (1), however, the diagnostic rate remains low. Presently, the diagnostic rate in developed countries including the UK is as low as 20-50% and only 10% in developing nations despite accounting for 60% of dementia patients (2). The prevalence of AD is expected to double every year and the economic burden for undiagnosed cases is great (3; 4). Research shows that patients who are diagnosed and treated have lower rates of institutionalisation, lower requirements for centralised resources and better chances of survival (3). How do we then achieve early detection and diagnosis if more than half of the people with dementia, the late stage of AD, are yet to receive one? This commentary describes the value of the modern diagnostic criteria for AD and the advantage of incorporating cheaper less invasive biomarkers examination with the clinical features of AD to improve the diagnosis of patients at the earlier stages of disease development.

The signature of AD includes five established biomarkers indicating the presence of the disease pathology in the brain of an individual with AD. These biomarkers are introduced by the extensive deposit of extracellular plaques of amyloid βeta (Aβ) peptides and intracellular neurofibrillary tangle (NFT) made up of phosphorylated tau (p-tau), low levels of aβ42 (**Figure 1**), increased concentration of total tau (t-tau) and phosphorylated tau (p-tau) within the cerebrospinal fluid (CSF), decreased glucose metabolism in the brain and cerebral atrophy (5;6;7).

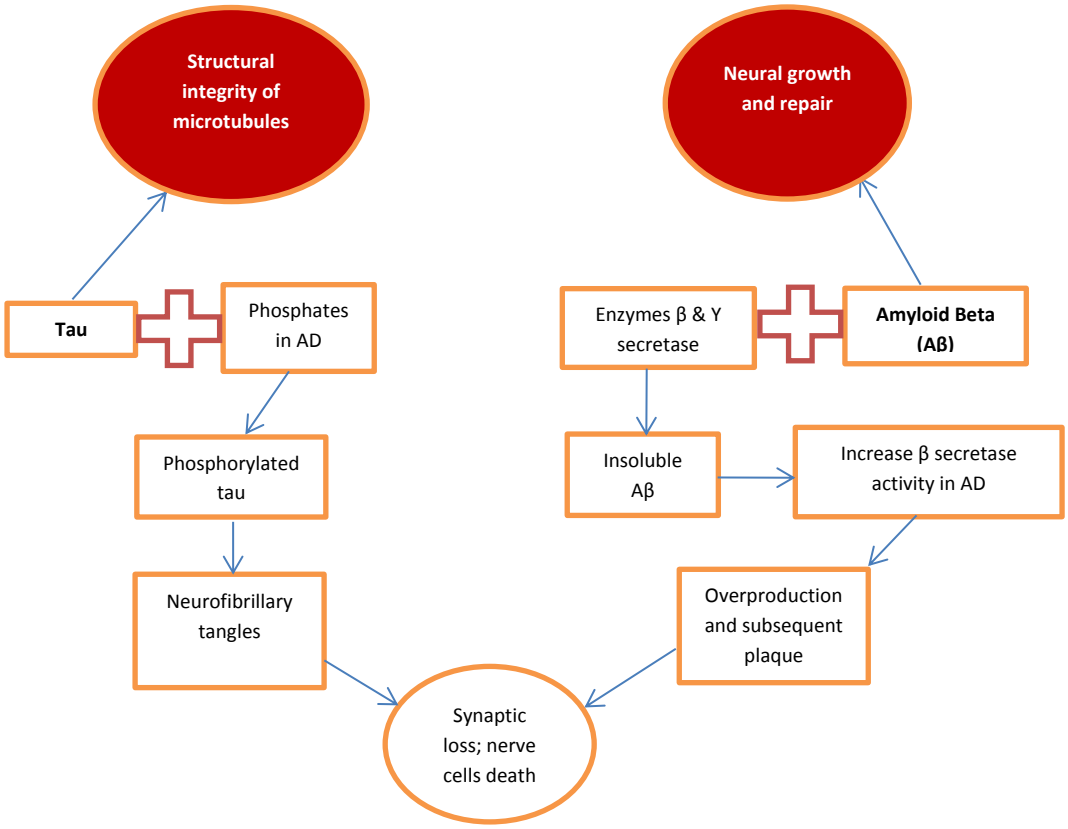

Figure 1. This is a schematic showing the main two biomarkers of AD (Tau and Amyloid Beta), their functions within normal cells; their interactions with other substances that lead to neurodegeneration and subsequent AD.

Other blood-based biomarkers have shown the potential to identify the disease at an early stage. For instance, ten different phospholipids found in the CSF were shown to be low in older individuals with impaired memory as compared to those with normal cognition, who within five years, converted from being healthy to having AD (8).

Other biomarkers identified through their association with AD pathology include neurogranin, a postsynaptic protein that binds with calmodulin at decreased calcium levels; neurogranin regulates synaptic signalling (9), which is significantly increased in AD. It is understood to correlate with a quick and sudden change in cognition at clinical follow-up in those with AD (10). Another protein is the neurofilament (NF), a prominent axonal cytoskeleton protein in neurons that maintains neuronal calibre; two (NF light -NFL and NF heavy-NFH) of its four subunits (NF-light, NF-medium,

NF-heavy, and alpha-intermexin) have been shown to increase in the CSF of those with AD (11).

These biomarkers are internal indicators that can indicate the presence of AD through laboratory tests or imaging tests; they assist in identifying the disease-related changes in the brain even before the stage of dementia or severe memory impairment (12). These biological markers are important in the diagnosis of AD alongside other measures like the history, features, clinical observations and other neurological tests.

However, the detection of biological markers is expensive, invasive and limited in availability. This is evidenced by the fact that the diagnostic criteria are still inaccessible in clinical practice, which is often the first point of contact with AD-affected patients. The challenges include the application of new technologies for diagnosis due to their invasive nature of biomarkers examinations, availability of time and expertise that is limited to some specialist centres, as well as the associated cost (13). In addition, the streamlined provision of healthcare means that doctors/healthcare providers no longer deliver the continuity of care needed to observe health deterioration, have inadequate training and/or are limited by insufficient resources (time, equipment) to make a diagnosis in the primary care setting (14). The diagnostic criteria have, however, taken into consideration the variability of the early symptoms and the overlap between AD and other similar conditions. We have previously shown that neurological and depressive behaviours are an early occurrence in early-onset AD while depressive and cognitive symptoms in semantic memory and conceptual formation were signs of late-onset AD (15).

The modern requisites for an early diagnosis of AD include clinical and research criteria. The clinical criteria are inexpensive instrumentation, while the research criteria make use of biomarkers, examined in clinical settings as part of research and/or clinical trials. These guidelines involve psychometric tests that identify the severity of the disease's abnormal cerebral aggregation. This is evidenced by fibrillary Aβ and hyper-p- *tau* in CSF and positron emission tomography (PET) brain imaging.

The leading biomarker measurement tests that are invasive, costly and not readily available in most first point of contact facilities include PET and Magnetic Resonance

Imaging (MRI), Computed Tomography (CT); Transcranial Magnetic Stimulation (TMS) in research centres (**Table 1**); however, Electroencephalography (EEG) and Electrovestibulography (EVestG) are both available in research and clinical practice (1;13). The current 'gold' standard for preclinical AD diagnosis is PET imaging or MRI in combination with CSF assays; while the CSF biomarkers are widely available in clinical research and practice, they are plagued by biases between laboratories, are invasive and need a trained specialist for the procedure to be performed safely (16).

Table 1: Biomarkers and related examinations: *The table indicates various biomarkers and their corresponding invasive and less invasive biomarkers examinations, their benefits and disadvantages as well as the corresponding cost for each test.*

Biomarker examination	**Biomarker**	**Benefits**	**Drawback**	**Associate cost (£)***
CSF Array	Aβ42 and p-tau peptides181	Widely available in both research and clinical settings	Biased and variability across laboratories, need trained specialist and a high-risk procedure	NPD
PET Scan	Brain imaging for reduced electrical activities in the cortical region	Highly sensitive and accurate	Expensive, invasive and limited in availability	689-795
MRI	Atrophy of the region of interest or hippocampal atrophy	Highly accurate and reliable	High risk, time-consuming, not readily available	199-363
CT Scan	The anatomical structure of the brain	Greater variability and precision	Expensive and limited in availability	450-600
EEG	Electrical signals in the brain	Generally safe and accurate	Need specially trained specialist	150-2500
Blood-based biopsies	Specific protein and microRNA (miRNA) concentrates	Reliable, less expensive and invasive, minimal risk and proxy for molecular evaluation, could be used for mass screening	Standardisation and regulations	Routinely done in the clinics
Saliva-based test	Non-coding RNAs in saliva, Iron-binding protein lactoferrin (Lf),	Non-invasive and expensive, readily available and compete positively with PET Scan	Less investigated	£15 and above
Liquid and gas chromatography-ion/mass spectrum measures	Plasma Aβ and p-tau 181	Mass screening, perform better with high accuracy	Regulation and standardisation	NPD
Retinal digital photographs imaging and examination	Amyloid beta, structural changes in the retina	Highly accurate, less invasive and expensive and applicable for mass screening	Standardisation of procedure	NPD

NPD: Not known, not in the public domain

A clinical support system for the early diagnosis of AD can be achieved through the use of computerised alerts, clinical guidelines, patients 'data' reports, reference information, documentation templates, automated historical comparisons, artificial intelligence and diagnostic tests (13). An example of such a support system is the PredictND, an automated and visual clinical decision support tool that combines information from multiple diagnostic tests (17). The tool has shown high accuracy in differentiating AD from other types of dementia and their controls, with a confidential measure for classifying subjective cognitive decline (SCD) or progression from MCI to AD dementia. This is because it combines information obtained from multiple diagnostic tests including neuropsychological inventory tests (NPI), MRI and CSF samples to achieve a diagnosis (17;18).

Apart from the fact that PredictND combines multiple tests especially those that are expensive, invasive and scarce in clinical practice, the PET imaging datasets need labelling time for the machines and the tool does not reflect the past historic context of current samples (13). The machine learning options are based on data collected from sophisticated tests and the AD neuroimaging (ADNI) database (16). Hence for economic and technical expertise reasons, this is not the option of choice for the clinical settings.

While the application of the new diagnostic criteria involving the use of sophisticated biomarkers examinations (1) is inaccessible in clinical practice, some options should be considered especially with the increase in prevalence and low diagnostic rates. These include the blood-based liquid plasma biopsies that have shown to be reliable for diagnosing AD. The procedure, which is not expensive, is considered less invasive and used as a proxy for molecular evaluation (19; 20). This can be reliably used in primary care in addition to other non-invasive and cost-effective diagnostic tools.

Additionally, oral samples including samples of saliva provide a promising source of non-invasive biomarkers that have been less investigated and compete well with the more expensive tests for biomarkers examined using PET scans (21; 22). Iron-binding protein lactoferrin (Lf), which is present in body fluids, is a promising biomarker candidate for AD diagnosis and MCI, as it indicates early antiviral activity

and is decreased in the saliva of patients with AD (21). Its levels have been shown to correlate with positive PET results.

Furthermore, breath-based examination using gas chromatography—ion mobility spectrometry (GC-IMS) has also been shown to be a reliable non-invasive and less expensive diagnostic measure for AD compared to the current diagnostic criteria. This is because the technique distinguishes AD patients from controls with high sensitivity and specificity, even in the presence of confounders such as age, gender, smoking and alcohol consumption that each influences the breath content (23). This could confidently be considered with other measures such as the signs and symptoms and NPI test (17; 18).

The neuropsychological examinations and nanopsychological testing have shown the suitability for use in conjunction with other diagnostic measures clinically. For example, the new nanopsychological testing tool is a touch screen computer-automated neuropsychological test battery. The testing tool measures neurodegeneration in the dementia stage and has been shown to be an accurate and clinically good diagnostic measure to use in this late stage of AD (17).

Other primary clinical diagnostic measures that form the basis of health science and are not fully emphasised in the current diagnostic criteria are the impairments associated with AD or the signs and symptoms. These impairments include:

- The olfactory impairment. There is an association between the loss of smell and taste that precede the dementia stage, which has been demonstrated to be an early symptom (24; 25). However, there is no standard olfactory test(s) available in clinical practice to support the diagnosis of AD.
- Vision changes seem to be overlooked in the early diagnosis of AD. Researchers (26; 27) have identified that the retina structural changes in examination predict AD early as well as the rate of progression. The retina, which is a sensory tissue that lines the back of the eye, converts light into a set of electrical signals and relays these signals to the brain along the optic nerve, it has demonstrated a link between cognitive capacity and visual acuity/ clarity (28). While structural changes like the shrinking of the retinal neurofibrillary layer (axon and ganglion cells that make up the optic nerve) have been identified in the late stage of AD, non-cognitive activities including visual perception and processing are affected at

the early stage of AD (29). This is an indication of neuropathy degeneration at the early stage of the disease. Hence, a good measure for clinical diagnosis is the retinal examination.

- The retinal examination consists of digital retinal photography; a computer-based-semi-automated technique that accurately quantifies retinal arteriolar central reflex and visual width similarity concerning the relationship between the microvascular health in the retina and the brain (30). Notwithstanding, even though the test has exhibited high sensitivity and specificity for the diagnosis of AD, it is not readily available in clinical practice.
- Speech impairment has also been well noted in early AD including verbal fluency and word-finding difficulty while separating MCI and controls; AD and non-AD (31). A communicative difficulty is a sensitive tool that could adequately be used for the early diagnosis of AD. This is exhibited in the form of narrative discourse and production deficit identified in a small cohort (32). As speech impairment is detectable in the NPI test, it has to be emphasised as a contemporary measure to support the diagnosis in clinical practice; this is because it presently lacks clear guidelines.
- Tinnitus, a false perception of sound without any external sound source is associated with altered pathways of auditory perception (33). While it is a comorbidity of hearing impairment, its sensory processing has also been associated with some cognitive functions such as learning, memory, behaviour, concentration and as a neurodegenerative soft sign, including AD in retrospective studies in small and large databases respectively (33; 34; 35). The correlation between this symptom and AD occurs early in the disease process and indicates the presence of AD with high accuracy.

Understandably, there are views that AD pharmacological treatments are costly, risky and with minimal benefits (13); however, there are non-therapeutic interventions that have been developed and are still being studied that can improve the course of the disease (36). The current diagnostic criteria for AD are based on biomarkers examinations or machine learning and pattern recognition techniques that have made the diagnosis at the MCI level possible in the research settings. It is also understandable that the clinical diagnosis of AD is insufficient with the individual

self-reported symptoms and the assessment of behavioural, functional and cognitive status through NPI tests especially in the pre-symptomatic phase.

However, the diagnosis at the pre-symptomatic stage can only be achieved for individuals who have been identified as being at risk of the disease either through genetics, age or exposure to specific environmental factors. Otherwise, the current diagnostic measures are retrospective using databases from PET scans, CSF or MRI of debilitated individuals; at best, leading to the diagnosis of AD at the later stages of the disease. It remains to be seen if PET scan, MRI and CSF or the PredictND tool could be applied in the primary care setting.

False-positive and negative are not unimportant in the early diagnosis of AD, especially with the less invasive, readily available biomarkers examinations. While the more sophisticated research-based biomarkers examinations present high accuracy and reliability in diagnosis, the clinical based less invasive and cheaper biomarkers examinations have equally shown high sensitivity and specificity. For instance, the blood-based examinations have reported a sensitivity of 90% and the combined use of structural-based CSF biomarkers Aβ and tau excluded false-positive cases with a specificity of 97% (37). Before then, the blood biomarker examinations accurately identified AD several years before the clinical diagnosis with a likelihood positive ratio of 7:9 (38), indicating their high accuracy.

Hence, for those that are at risk, we suggest that the current clinical procedure should include the signs and symptoms that are associated with the earliest stages of AD (25; 34; 27; 35) even before memory loss. For example, olfactory symptoms, auditory symptoms and vision changes, in association with the less invasive examinations of biomarkers have shown to be as effective at confirming an AD diagnosis as PET scans. These can be used together with plasma or saliva biomarkers tests that are less invasive, cheaper and readily available as a diagnostic measurement for the clinical settings. This will give physicians the confidence to make an earlier diagnosis within clinical practice and where there are disagreements, the more sophisticated tests available in research centres can be used for confirmation to reduce the cases of false-positive and negative cases.

The signs and symptoms, and blood-based biomarkers should continue to be validated, adding in saliva biomarkers for extra reliability, while taking advantage of

the retinal biomarkers (including changes in retinal nerve fiber layer; thickness; degeneration, reduction in blood flow with other vascular changes and the presence of Aβ1–42 and *p-tau*) (26; 27). This is because they are each non-invasive, cost and time-efficient with low risk, and relevant at the early stage of the disease. These biomarkers could also support the diagnosis of AD in the developing world where the prevalence is high and there is a decreased chance of healthcare providers or citizens being able to afford to offer or undertake tests that support the current diagnostic criteria.

The chances of false-positive and negative will be reduced with standardisation of the samples, correlation between analysis threshold, values and storage time. This is exemplified by the significant differences in the results of a multi-centre study on the deregulation of four microRNA (miRNAs) in the CSF of individuals with AD (39) which were shown to be caused by issues with standardisation. If, as reported by Alzheimer's Disease International (4), that someone develops dementia every three seconds then urgent measures are needed to improve the diagnostic rate for AD and facilitate timely intervention. Therefore, we are calling for a concerted effort to identify and facilitate the use of a predictive and a diagnostic tool that can easily be applied in the clinical setting to support the early diagnosis of AD. This is possible with improved primary care-based routine electronic health records (EHR), and the advance of machine learning (ML) techniques.

Keywords

Alzheimer’s disease; Early diagnosis; Diagnostic criteria; Biomarkers examinations; early signs and symptoms; Less invasive.

Abbreviations

AD: Alzheimer's disease; ADNI: AD Neuro Imaging; CSF: Cerebro Spinal Fluid; CT: Computed Tomography; EEG: EHR: Electronic Health Records; Electroencephalography; EVestG: Electrovestibulography; GC-IMS: Gas Chromatography—Ion Mobility Spectrometry; LT: Lacto Ferrin; MicroRNA: Micro Ribonucleic Acid; ML: Machine Learning; MRI: Magnetic Resonance Imaging; NF: Neuro Filament; NFT: Neuro Fibrillary Tangle; NPI: Neuro Psychological Inventory; PET: Positron Emission Tomography; SCD: Subjective Cognitive Decline.

Declarations:

Authors' contributions

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