Diagnose it yourself: will there be a home test kit for Alzheimer's disease?

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Practice points

- General practitioners are faced with old age patients complaining about decline of cognitive function every day. Timely and accurate diagnosis of neurodegenerative processes potentially leading to dementia is essential for implementing preventive and therapeutic measures.
- The most common cause for dementia is Alzheimer's disease (AD). Today's gold standard for the diagnosis of AD is high in effort and cost. An *in vivo* diagnosis with 100% accuracy is still not possible.
- There is a demand for AD testing systems with wide accessibility that give reproducible and reliable results, ideally for use at home.
- Novel techniques are focusing on biomarkers in peripheral specimens, including blood, cells, saliva or urine, yielding conflicting results.
- Thus far, the most promising approach are blood-borne biomarkers targeting analytes usually quantified in cerebrospinal fluid like tau or amyloid. These markers measured in blood can yield reliable and valid results. However, inter- and intra-laboratory variability is still high.
- Other bloodwork approaches are targeting neuroinflammatory and neuroprotective biomarkers, which are known to be involved in neurodegenerative processes such as AD. Another promising marker might be NfL measured in blood serum or plasma.
- Due to technical limitations, an AD test for sole use at home is unlikely at this point. However, a testing kit for taking blood at home to send directly to a laboratory which in return sends the results with an interpretation, seems technically feasible in the near future.
- Based on the available literature, a panel of *APOE* genotyping, amyloid-beta 1-42/1-40 ratio, p-tau and NfL quantified in the blood provides a promising biomarker combination.

Alzheimer's disease is the most common neurodegenerative process leading to dementia. To date, there is no curative approach; thus, establishing a diagnosis as early as possible is necessary to implement preventive measures. However, today's gold standard for diagnosing Alzheimer's disease is high in both cost and effort and is not readily available. This defines the need for low-effort and economic alternatives that give patients low-threshold access to testing systems at their general practitioners or even at home for an independent retrieval of a biologic specimen. This perspective gives an overview of established and novel approaches in the field and speculates on the future of test strategies eventually technically implementable at home.

Lay abstract: Alzheimer's disease is a common cause for dementia. While there is no cure yet, finding a diagnosis as early as possible is necessary to slow down worsening of cognitive abilities as much as possible. The commonly administered diagnostic tools for Alzheimer's disease are high in both cost and effort. This emphasizes the need for low-effort and economic alternatives, that give patients a low-threshold access to testing systems at their general practitioners or at home in a self-application. This perspective gives an overview of today's diagnostic standard and reviews novel approaches in the field. It also speculates on the future of strategies that might potentially be suitable for taking a diagnostic test at home.

Tweetable abstract: Will it one day be possible to diagnose Alzheimer's Disease at home? This perspective article is discussing techniques, which might make it feasible in the future.

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Keywords: Alzheimer's disease • biomarkers • dementia • diagnostics • novel techniques

Millions of people worldwide are affected by neurodegenerative diseases that can lead to dementia [1], first and foremost by Alzheimer's disease (AD). This has large-scale social and economic implications and poses a heavy burden individually for the patients themselves and their caretakers and the whole community in the form of finance needed for diagnostics and treatment of the disease. Thus, there is a need for an easily accessible, minimally invasive, economic and reliable diagnostic test capable of validly detecting the presence of AD earlier than is currently the case. According to a survey conducted by the 2019 World Alzheimer Report [2], 95% of around 70,000 respondents think they could develop dementia in their lifetime and 82% would take a genetic test to learn about their risk. Apart from the rare autosomal dominant familial AD, which only accounts for less than 1% of AD cases [3], the sporadically inherited *E4* variant of *APOE* gene has been identified as increasing the risk for AD [4]. Being aware early of having an elevated risk for developing dementia due to AD and subsequently making lifestyle changes can delay cognitive worsening in subjects at risk for dementia [5]. There is evidence showing the positive and lasting effect of primary and secondary prevention in AD [6]. By taking preventive measures, there could be several millions of fewer patients and their caregivers burdened by the disease [7]. Furthermore, accurate diagnostic measures, in general, are in demand not only by the wider public but also by pharmaceutical companies for various other reasons. So far, no causal therapy against AD is available, but the hunt for adequate curative treatment is continuing. Due to the not fully understood etiology of AD, a wider range of biomarkers might help to determine the individual risk and nature of a patient's disease. This is not only for attaining an easy and economical diagnostic tool identifying participants at risk to include them in clinical trials but also gaining more insight into the causes of the disease to develop a causal treatment eventually.

To understand today's approaches of biomarker-based diagnosis of AD, the currently established hallmarks of AD need to be considered. The characteristics of AD, the most common cause for dementia, are extracellular amyloid plaques and intracellular neurofibrillary tangles [8]. It is believed that intracerebral cellular changes, especially the accumulation of amyloid, occur up to 20 years before the onset of clinical symptoms, including loss of memory and decline of orientation or speech [9]. The clinical course of AD is usually a slow but gradual process: it was shown that subjective cognitive decline, defined as the subjective experience of worsening cognitive performance among cognitively normal older individuals, can represent an at-risk stage of AD [10,11]. Further down the progress, when clinical symptoms become objectifiable but are not as pronounced as to impair activities of daily living and with neurobiological evidence for underlying AD pathophysiology, the syndrome of mild cognitive impairment (MCI) due to AD can be diagnosed [12]. As soon as a patient needs assistance in their daily routines, the clinical stage of dementia is reached.

Reaching the causal underlying alterations for AD in the brain is hindered by different factors, one important being the blood–brain barrier (BBB). The BBB is a semipermeable border of endothelial cells [13] supposed to protect neurons from pathogens circulating in the blood, while at the same time allowing transport of needed elements like nutrients, glucose or water [14]. However, this highly effective system makes it difficult to gain access to the intracerebral pathological changes and its substrates needed for diagnosing neurodegenerative processes due to the BBB preventing the passage of molecules between the brain and blood system [15].

Despite these challenges, in recent years, there is more and more evidence from peripheral markers that systemic infection and inflammation might play a causal role in AD-related neuropathological processes [16,17]. This puts in focus potential biomarkers to measure these changes, where not only a single marker might be relevant but rather a combination of several markers [18,19]. In general, it is by now already possible to detect a number of biomarkers in less than 0.5 ml of blood [19,20]. Yet, as of now, these analyses require a great amount of effort and time as well as technical equipment and expertise.

In this Perspective article, we give a review on the existing gold standard of diagnosing AD as well as discuss innovative technologies using specimens such as saliva or peripheral cells. We put a focus on novel techniques that one day might be suitable for use at home, without the necessity of seeing a physician to retrieve a biological specimen for biomarker analysis. Table 1 gives a three-tier graphical overview of the biomarkers evaluated in this article. It recapitulates the cost and effort to analyze the respective markers. Furthermore, we summarize the diagnostic accuracy and speculate on the potential at-home-use.

Table 1. Graphic evaluation of the potential usability of different novel Alzheimer's disease diagnostic methods.

	Analytical cost and effort	Diagnostic accuracy	Potential at-home-use
Blood Tau	0	+	0
Blood amyloid	-	+	0
Blood NfL	0	+	0
Blood neuroinflammatory markers	-	+	0
Olfactory epithelium	+	0	0
Saliva	0	0	+
Buccal cells	0	0	+
Urine	-	-	+

Note: We defined the range by the grades '+' (favorable), '0' (undecided) and '-' (unfavorable).

Today's gold standard

Today, the diagnosis of AD is still complex, even for specialized experts. Depending on the stage of the disease – earlier or later – a multitude of information is needed. However, compared with 20 years ago, diagnosing probable AD has made extensive advances regarding cost and effort. Thanks to those advances, gathering replicable evidence for underlying AD-related neurodegeneration is by now more common.

At specialized centers, neuropsychological assessments and clinical information are the basis to determining different stages of cognitive decline [21,22]. Additionally, there are various methods available to obtain evidence for an underlying AD pathology in cognitively impaired patients. The more biomarkers and further clinical information are combined, the more accurate the diagnosis [23]. Evidence for AD-typical pathophysiological changes in the brain plays a key role in recent perceptions of a diagnostic strategy: the updated 2018 research framework for the definition of AD is based on biomarkers [24] and gives a biological definition of the disease. According to this framework, one speaks of AD when there is evidence for both an abnormal phosphorylated Tau (T+) and amyloid-beta 1-42 (A+) level quantified in cerebrospinal fluid (CSF) or by positron emission tomography (PET) [25,26]. However, both of these methods are solely available in specialized centers. Furthermore, they may be perceived as highly invasive by some patients (lumbar puncture) or patients face a small dose of radiation for a very costly technology (PET). Another important source of information in the diagnosis of AD are brain MRI scans [27]. This is especially needed to assess patterns of brain atrophy and rule out other causes for cognitive decline like vascular damages [28] or brain tumors [29]. While this technology is widely available, it is cost-intensive, up to twice the amount of a computer tomography (CT), which among other disadvantages, lacks the high resolution of MRI scans. Other approaches may add to diagnosing AD, such as EEG or event-related potentials [30] or the examination of eye movement and cognitive processing [31]. Yet again, these techniques require highly specialized personnel and much time per patient and thus are not readily available to the public.

Thus, it is evident that there remains a big gap between the desirable research-based diagnostic measures and the factual community-based diagnostics that outpatients are able to access at their general practitioners or even at home. Furthermore, depending on the number of diagnostic tools used, the diagnostic procedure can be a heavy burden on the patient, their caregivers, and not least on the social system covering the cost.

Blood

Today, one of the most pursued approaches in the field of biomarkers for AD is the utilization of blood or blood components. Currently, most research activities into blood-borne biomarkers for AD focus on the biomarkers that were established as gold standard for the diagnosis of AD in CSF: amyloid-beta and Tau species. However, there are continuously evolving approaches to find other markers that might reflect neuropathological processes underlying AD, such as small metabolites. For example, sphingolipids have been found to be associated with measures of progression during preclinical and prodromal AD and are also discussed as new targets for developing new treatments against the disease [32,33].

Blood – Tau

While the quantification of total-Tau in the blood may not be the most promising approach [34], the levels of different phospho-Tau species in blood might be more suitable to indicate neurodegenerative processes in the brain. Examining the value of plasma p-tau181 with a newly developed ultrasensitive blood immunoassay, evidence

shows a gradual increase along the Alzheimer's continuum: young adults and cognitively healthy adults had the lowest concentration, while the highest concentrations were found in amyloid β -positive MCI and AD groups [35]. Furthermore, plasma p-tau181 showed a high differential diagnostic ability from brain diseases like frontotemporal or vascular dementia. There is more evidence for the potential diagnostic utility of plasma p-tau181 [36–38] and plasma p-tau217 [39], showing positive associations with PET and CSF tau biomarkers and high diagnostic value to differ between AD patients, patients with neurodegenerative disorders other than AD and healthy controls.

Blood – amyloid

Recent work suggests that quantification of amyloid in blood products might have diagnostic utility. Data suggests that maximally standardized pre-analytical and analytical conditions are the key to reliable measures of amyloid in blood [40,41]. Along this line, data from fully automated Elecsys immunoassays (Roche Diagnostics, Rotkreuz, Switzerland) predict amyloid-beta-positivity (defined by CSF values) with the means of blood plasma amyloid-beta and showed promising results in doing so with AUCs between 0.8 and 0.86 [41]. Also, there is evidence showing that plasma amyloid-beta concentrations measured by mass spectrometry have positive correlations with cerebral β -amyloidosis [42–44] in the context of AD. Other complex methodological approaches confirm the general suitability of blood amyloid for the diagnosis of AD. The isolation of neuronal-derived exosomes from blood, subsequent transmission electron microscopy and western blot [45] showed results comparable to those for the quantification of CSF biomarkers in subjects at different disease stages. There is even evidence for secondary structure changes in amyloid-beta in blood. It was found that this biomarker when using an immuno-infrared sensor, may detect AD several years before clinical diagnosis with a positive likelihood ratio of 7.9 [46].

Blood – neuroinflammatory & neuroprotective markers

A promising approach outside of the classical markers amyloid and tau in the blood is analyzing levels of neuroinflammatory and neuroprotective serum or plasma protein markers. Since neuroinflammatory pathways become deregulated decades before the clinical onset of AD, targeting these mechanisms might be a novel diagnostic and even therapeutic approach [47]. There is evidence that the combination of the six BDNF, IGF-1, VEGF, TGF- β 1, MCP-1 and IL-18 quantified in blood serum can indicate the presence of AD at the dementia [19] and [48] MCI stage and can serve as potential diagnostic tools by combining the results with an algorithm. There are more data showing associations between neuroinflammatory markers in serum such as IL-8 and CSF p-tau181 [18] as well as clinical AD [49].

Blood – NfL

NfL is an intra-axonal structural protein that is detectable not only in CSF but also in blood plasma and serum and indicates neuroaxonal injury [50]. Plasma (or serum) levels correlate with CSF NfL levels and are increased in both sporadic and familial AD [50]. Plasma NfL is associated with cognitive, biochemical, and imaging features of AD [36,51]. Furthermore, a rapid increase of NfL is associated with faster worsening in global cognition [52]: plasma NfL levels increased over time in association with baseline CSF biomarkers, in other words, low A β 42 as well as high total tau and high phosphorylated tau levels.

Olfactory epithelium

It is well described that olfactory dysfunction increases with the severity of AD [53], and there is immunohistochemical evidence of tau pathology in the olfactory bulb and nerve that correlates with the severity of AD [54]. There are contradictory reports, but there is evidence for a higher expression of amyloid-beta and paired helical filament tau in the olfactory epithelium of AD patients post mortem in contrast to controls and also to subjects with other neurodegenerative diseases [55]. The methoxy-X04 derivative BSC4090, a fluorescent ligand designed to target neurofibrillary tangles in AD in olfactory mucosa, might even allow for early differential diagnosis [56]. Thus, smears from the olfactory mucosa might be promising specimens to look into when developing easily applicable approaches for diagnostic implementations.

Saliva

The examination of saliva components sheds light on a different potential novel biomarker in the detection of AD. The analysis of markers like cortisol [57,58], different metabolites [59,60] or markers of oxidative stress [61,62] yields varying results. However, these findings show that biomarkers outside of amyloid-beta and Tau might be worth

further investigation. Yet, when analyzing the established AD biomarkers amyloid-beta and Tau, studies in saliva come to different conclusions. Changes in amyloid could be found in different cohorts [63–65], while other studies could not confirm these findings [66,67]. Like in blood, total-Tau alone might not be the most suitable marker in saliva [68], whereas phospho-Tau might improve diagnostic accuracy [66].

Buccal cells

There is evidence that examining buccal cells for biomarkers in AD might be a promising approach. Apart from amyloid-beta and Tau, markers of interest in buccal cells are telomeres [69–72], DNA content and structure [73–75] or combined biomarker panels [76], showing the ability to differentiate between healthy control subjects and patients suffering from MCI and AD. Different studies were able to confirm correlations between buccal cell-derived biomarkers and clinical AD. Furthermore, it was shown that there are correlations between the presence of p-Tau, t-Tau and amyloid-beta in buccal cells with CSF and neuropsychological data [74,77,78]. However, as with other novel approaches, results are differing, not least due to lots of different techniques applied and varying biomarkers examined.

Urine

Promising evidence of urine biomarkers for the diagnosis of AD in humans is scarce. Two studies found the proteins SPP1, GSN, IGFBP7 [79], and AD7c-NTP levels [80] in urine to significantly differ between AD patients and healthy controls.

Conclusion

The listed scientific data shows a variety of new approaches that are pursued to facilitate the diagnosis of AD. However, many challenges need to be overcome until any of these approaches can be used for routine diagnostics.

Future perspective

Since discovering the diagnostic value of amyloid-beta and Tau in CSF, there have been attempts to find biomarkers or signatures indicative of AD outside of these established ones. Research into these markers has again highlighted that AD is a disease expressed in very heterogeneous biomarker patterns [81] and clinical features [82]. This complicates finding a singular diagnostic method encompassing this complex process taking place in the brain. It is reasonable to believe that in the future there will be differential diagnoses of AD subtypes [83], each of which might be defined by alterations in diverse biomarkers – yielding adaptive curative approaches. However, even assuming that various AD subtypes can be agreed upon, there are various obstacles to overcome until there is a test that can detect AD in a readily available bodily specimen. From experience gained with the classical CSF biomarkers, a crucial point are the pre-analytical conditions: many studies have shown that a lack of standardization in sampling and processing leads to varying results [84]. Furthermore, different cohorts examined with the same methodology yield different results [85], which yet again illustrates the heterogeneity of the disease and the need for maximal standardization of pre-analytical and analytical conditions. Besides this, there are method-specific challenges that may lead to the measured values not actually mirroring the respective specimen's biomarker level, for example, in immunoassays [86,87].

Thus, the reliability and reproducibility of a potential home test kit is hard to achieve, yet it is needed for enabling a readily interpretable result. Further multicenter studies will need to be conducted to evaluate which method has the lowest inter- and intra-subject variability while at the same time being low in cost and effort. The same holds true for the assessment of inter- and intra-laboratory variability. In these trials, correlations of novel biomarkers will compulsorily need to be undertaken with the established gold standard biomarkers like CSF and PET and in the best case with post-mortem histopathologically confirmed AD cases. At the same time, it needs to be kept in mind that amyloid and Tau *in vivo* biomarkers only have a specificity and sensitivity of a maximum of 90% (for amyloid PET) and 53–83% (for CSF biomarkers), respectively [88]. Also, biomarker levels, especially in neurodegenerative diseases like AD, have to be standardized according to age and potentially sex [89]. For instance, it is a well-replicated finding that CSF tau levels increase naturally with age, without necessarily reflecting a pathological process [90]. This trend could also be shown for NfL, which increases around 3% per year in CSF of cognitively healthy subjects [91].

Based on the evidence available so far and taking ethical considerations into account, we do not see a home test kit with a dichotomous AD-negative or AD-positive test result in the foreseeable future. From an ethical point of view, the interpretation of medical test results without professional assistance and the potential consequence on a

patient's well-being is a major challenge, given that health literacy is limited in the general population [92]. However, self-testing kits analyzing genetic information are available, which among other data give information about one's *APOE* status, thereby already potentially indicating an increased risk for AD.

Much like already available HIV test kits for at-home-use [93], similarly, in the field of AD we can imagine a testing kit, where a small amount of blood from the fingertip is taken and sent to a laboratory. It would then be processed, and subsequently, the results and an interpretation of the outcome, as well as a recommended course of action are given. Looking at current evidence on the technical feasibility of AD biomarkers, we can see a panel of *APOE*, amyloid-beta ratio 1-42/1-40, p-Tau and NfL in blood as a promising combination.

However, for the near future, we believe that these results would give information of being at a higher risk for AD compared with the normal population, rather than a formal diagnosis. With this knowledge, one may implement secondary preventive measures or deliberate with a physician if further diagnostic procedures or treatment is justified. For persons worrying about declining cognitive functioning, we can see this hypothetical test as a method for the exclusion of AD and thus search for other reasons for cognitive decline in old age, such as vascular etiology [28] or depression [94]. Subsequently, patients and general practitioners may take action to implement according to treatment. For earlier stages of AD, even before the onset of subjective worrying about cognitive decline, there will need to be more research focusing on these subjects before a meaningful test can become available.

Author contributions

F Menne and CG Schipke were responsible for conceptualization, literature research and writing of the manuscript.

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