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Commentary

Spitting image: can saliva biomarkers reflect Alzheimer's disease?

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An easily accessible and non-invasive biofluid test to characterize the pathological hallmarks of Alzheimer's disease (AD), amyloid−β and tau, has been long sought after. Such tests could assist in the differential diagnosis of cognitive decline and, in the long pre-symptomatic phase of AD, highlight those who might benefit the most from therapeutic intervention. Cerebrospinal fluid (CSF) for amyloid−β (Aβ42/40), total tau (*t*-tau) and phosphorylated tau (*p*-tau) have led the way in this regard, and are now incorporated into diagnostic criteria for AD. Yet, there is some reluctance towards CSF collection via lumbar puncture due to its alleged invasiveness or the specialism that it requires. In this context, the newly developed blood tests for the detection of amyloid−β and *p*-tau [1], which have been shown to be highly specific to AD pathology, and neurofilament light (NfL) [2], which is general biomarker for axonal injury, have taken center stage and have enormous potential for extensive application in clinical medicine.

Then, the question remains, can we go further than blood? Are other biofluids also an option? Although blood biomarkers are easier than CSF or position emission tomography (PET) to implement in clinical practice, they still have minor logistical challenges *e.g.,* venipuncture collection or controlled pre-analytics. A bio resource such as saliva may offer an even more simplistic alternative, which may be favored by patients or study participants. One could even envisage a home collection protocol for saliva for research studies, potential recruitment into drug trials or, in the recent epidemic events of 2020−2021, clinical monitoring when visitation is not permitted. However, saliva has to be unequivocally proven to be useful biofluid in a dementia setting before this can even be contemplated.

AD-related biomarkers have been reported in saliva, with varying results [3,4]. It is clear that detectable levels of t-tau [5] and NfL [6] have little association with clinical status or biomarker profiles. A recent report [7], which was subsequently replicated by the same researchers [8], recently demonstrated compelling evidence of decreased salivary lactoferrin (Lf) in AD patients. Lf is an iron-binding protein and expressed in all body fluids, and has a wide variety of physiological functions. Importantly, in the context of AD, Lf has been detected in senile plaques, neurofibrillary tangles and microglia from AD brains. Lf was found to be decreased in amyloid−β PET positive patients but not in amyloid−β PET negative healthy individuals or those diagnosed with frontotemporal dementia. Thus, Lf may represent the first highly specific salivary biomarker for AD.

In this issue of EBioMedicine, Gleerup and co-workers sought to replicate the potential use of salivary Lf in a mixed memory clinical population [9]. They recruited 222 participants, including healthy controls, as well as patients with mild cognitive impairment (MCI), AD and a mixture of non-AD neurodegenerative diseases, all with CSF Aβ42, *t*-tau and *p*-tau profiles. The results reported were not able to reproduce any aspect of the previous findings on salivary Lf. This included no significant change of salivary Lf across diagnostic groups even when normalized to the total protein content in the saliva sample. Furthermore, there was no association between salivary Lf and the "core" CSF AD biomarkers. In a novel approach, Lf concentration was measured in the CSF but did not correlate with salivary Lf concentration from the same patient. It is problematic that two studies with similar methodology, in terms of sample collection, statistical power and Lf detection can be so different in conclusion. This further emphasises the importance of reproducing research results on promising biomarkers in two or more independent clinical cohorts in the original publication [10]. In our mind, this also questions the validity and reproducibility of saliva collection, which is controlled primarily by the patient and not by the clinician (*e.g.* venepuncture or lumbar puncture), and should be examined further. Saliva production varies between major salivary glands, not only in its production but also its content. Unstimulated saliva, the preferred matrix in the aforementioned Lf studies, is predominantly produced by the submandibular

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gland, whereas stimulated saliva is produced mostly from the parotid gland. Thus, this saliva production could potentially be variable and sensitive to external stimuli. Lastly, saliva flow is affected in older adults. This maybe a direct consequence of ageing or indirect hyposalivation from medication use and it is often difficult for a demented individual to produce a sufficient sample for analysis [3] which speaks against it being an "easier" biofluid for clinical practice. In the proposed Lf studies [7–9], there is no insight into the effect of medical use or oral hygiene in the enrolled patients – this could have a large influence on the composition of the saliva matrix collected and subsequently Lf concentrations. The other outstanding issue to address is how a biofluid like saliva could reflect changes in central nervous system (CNS)-related processes. Abundant innervation, along which CNS-enriched biomarkers could travel, has been suggested as a potential route but needs to be proven.

Saliva is a rich bioresource which is easily obtained. The potential of self-collection is a huge attribute but maybe a large source of variability which simply does not apply to venepuncture or lumbar puncture. A combination of positive and negative reports has been published on a host of salivary biomarkers of interest to AD, namely, amyloid$-\beta$, tau, α-synuclein and this recent negative report of salivary Lf by Gleerup and co-workers fits this trend of uncertainty. This study clearly highlights the major need for experiments addressing clearance pathways of CNS biomarkers into the saliva, as well as standardization and consensus in the way we collect, process and store saliva if the scientific community are to take this biofluid seriously in neurodegenerative diagnostics.

Declaration of Competing Interest

NJA does not have any conflict of interest to disclose. KB has served as a consultant or at advisory boards for Abcam, Axon, Biogen, JOMDD/Shimadzu, Lilly, MagQu, Prothena, Roche Diagnostics, and Siemens Healthineers. KB has served at data monitoring committees for Julius Clinical and Novartis. HZ has served at scientific advisory boards for Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies and CogRx. HZ has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen. KB and HZ are co-founders of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.

Contributors

NJA, KB & HZ contributed equally in the writing this commentary.

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References

[1] Ashton NJ, et al. The validation status of blood biomarkers of amyloid and phospho-tau assessed with the 5-phase development framework for AD biomarkers. Eur J Nucl Med Mol Imaging 2021. doi: 10.1007/s00259-021-05253-y.
[2] Khalil M, et al. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol 2018;14:577–89. doi: 10.1038/s41582-018-0058-z.
[3] Ashton NJ, Ide M, Zetterberg H, Blennow K. Salivary biomarkers for Alzheimer's disease and related disorders. Neurol Ther 2019;8:83–94. doi: 10.1007/s40120-019-00168-1.
[4] Gleerup HS, Hasselbalch SG, Simonsen AH. Biomarkers for Alzheimer's disease in saliva: a systematic review. Dis Markers 2019;2019:4761054. doi: 10.1155/2019/4761054.
[5] Ashton NJ, et al. No association of salivary total tau concentration with Alzheimer's disease. Neurobiol Aging 2018;70:125–7. doi: 10.1016/j.neurobiolaging.2018.06.014.
[6] Gleerup HS, et al. Saliva neurofilament light chain is not a diagnostic biomarker for neurodegeneration in a mixed memory clinic population. Front Aging Neurosci 2021 In Press.
[7] Carro E, et al. Early diagnosis of mild cognitive impairment and Alzheimer's disease based on salivary lactoferrin. Alzheimers Dement 2017;8:131–8. doi: 10.1016/j.dadm.2017.04.002.
[8] Gonzalez-Sanchez M, et al. Decreased salivary lactoferrin levels are specific to Alzheimer's disease. EBioMedicine 2020;57:102834. doi: 10.1016/j.ebiom.2020.102834.
[9] Gleerup HS, Jensen CS, Hogh P, Hasselbalch SG, Simonsen AH. Lactoferrin in cerebrospinal fluid and saliva is not a diagnostic biomarker for Alzheimer's disease in a mixed memory clinic population. EBioMedicine 2021;67:103361. doi: 10.1016/j.ebiom.2021.103361.
[10] Mattsson-Carlgren N, Palmqvist S, Blennow K, Hansson O. Increasing the reproducibility of fluid biomarker studies in neurodegenerative studies. Nat Commun 2020;11:6252. doi: 10.1038/s41467-020-19957-6.