No association of salivary tau concentration with Alzheimer's disease

Nicholas J. Ashton [1, 2, 3, 4], Mark Ide [5], Michael Schöll [1, 6], Kaj Blennow [2, 7], Simon Lovestone [8], Abdul Hye [3, 4] and Henrik Zetterberg [2, 7, 9, 10]

[1] Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Gothenburg, Sweden. [2] Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden. [3] King's College London, Institute of Psychiatry, Psychology and Neuroscience, Maurice Wohl Institute Clinical Neuroscience Institute, London, UK. [4] NIHR Biomedical Research Centre for Mental Health and Biomedical Research Unit for Dementia at South London and Maudsley NHS Foundation, London, UK. [5] Periodontology/Oral and Mucosal Biology, Dental Institute, King's College London, London, UK. [6] Clinical Memory Research Unit, Department of Clinical Sciences, Malmö, Lund University, Lund, Sweden. [7] Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden. [8] Department of Psychiatry, University of Oxford, Warneford Hospital, Oxford. [9] Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, UK. [10] UK Dementia Research Institute at UCL, London, UK.

ABSTRACT – There is a need for an accessible biomarker that can complement current cerebrospinal fluid (CSF) and positron emission tomography (PET) biomarkers in an accurate and early diagnosis of Alzheimer disease (AD). Saliva is a rich source of potential biomarkers and proteins related to neurodegenerative disorders have been shown to be present in this matrix, including tau. In this study, we quantified salivary total tau concentration in 160 Alzheimer's disease (AD), 60 mild cognitive impairment (MCI) and 54 healthy elderly control (HEC) participants using ultra-sensitive Single molecule array (Simoa) technology. Salivary tau concentration in AD did not differ from MCI or HEC. In addition, there was no association of salivary tau concentration with neurophysiological assessment or structural MRI. Due to the large overlaps in concentrations between clinical groups, we conclude that salivary tau is neither a suitable biomarker for AD nor for cognitive impairment.

INTRODUCTION – The diagnosis of Alzheimer's disease (AD) remains primarily reliant on clinical assessment. Advancements in the latest decade have recognised the valuable role of both imaging (PET imaging of amyloid and tau aggregates) and core CSF biomarkers (Aβ42, t-tau and p-tau), which identify AD pathophysiology with high accuracy. However, both modalities have disadvantages when considering widespread implementation of a test for suspected AD in clinical practice or for participant selection for therapeutic trials.

A decrease in CSF Aβ42 is postulated to be the earliest biochemical change in AD [1]. However, CSF t-tau may be considered more clinically relevant and a disease intensity marker; the higher the concentration, the more intense the neurodegenerative process [2]. Using ultrasensitive measurement techniques, the protein can be measured in plasma and efforts have been made to clarify the role of plasma tau in AD. There is general agreement that concentrations are increased [3] but the overlap between clinical groups is larger than for CSF tau [4]. In addition, the correlation of plasma with CSF tau is weak [5]. Plasma tau concentrations, in contrast to CSF, may be confounded by the rapid degradation of tau in blood; the half-life of tau in plasma is hours [6] compared to weeks in the CSF (Sato *et al*., in press), but also by extra-cerebral tau mRNA and protein expression (Protein Atlas

Reference). It is possible that some CNS-derived proteins are eventually excreted into body fluids other than CSF and blood. The presence of tau in saliva has been demonstrated using mass spectrometry [7]. However, the relationship between salivary concentrations of tau and processes within the CNS is far from clear, and no conclusive data on disease association have been reported so far. In this study, we examined the diagnostic accuracy of salivary tau concentration for AD in dementia and mild cognitive impairment (MCI) patients as compared to cognitively normal control individuals. Furthermore, we investigated the relationship of salivary tau concentration with neurophysiological and MRI measures.

MATERIAL AND METHODS – Saliva samples of 160 AD, 60 MCI and 54 HEC participants were obtained from a single centre from the AddNeuroMed consortium (Kings Health Partners-Dementia Case Register (KHP-DCR)) [8]. Details regarding clinical diagnosis, cognitive assessments, *APOE* genotyping and magnetic resonance imaging (MRI) acquisition have been previously described [9]. Saliva samples were diluted 4-times and measured in duplicate for total tau using the commercially available Human Total Tau assay on an HD-1 Simoa instrument (Quanterix, Lexington, MA) [6]. Statistical analysis was performed by using IBM SPSS Statistics, version 25 (Armonk, NY, USA). Associations between salivary tau and demographic factors were assessed and a generalised linear model (GLM) corrected for the significant differences of age and years of education between in the diagnostic groups (Table 1). The differences of salivary tau concentrations between diagnostic groups were calculated by Analysis of variance (ANOVA). Correlations between adjusted salivary tau levels and MMSE and MRI measures were calculated using pearson *r*.

CORE DATA – The demographics for the study population are detailed in Table 1. Salivary tau was quantifiable in 96.6% of participants included in the study (LoD = 0.9 ng/L), with an average coefficient of variation of 11.5% for duplicate measurements. Salivary tau was not associated with age (r = 0.080, P = 0.190), years of education (r = -0.033, P = 0.586), sex (median, 9.6 ng/L for females versus 12.3 ng/L for males; P = 0.872) or *APOE* ε4 genotype (median, 8.1 ng/L in non-carriers versus 9.7 ng/L in carriers; P = 0.788). We observed a non-significant increase of salivary tau

concentration across diagnostic groups (median, 9.6 ng/L for HEC, 9.8 ng/L for MCI and 12.3 ng/L for AD; $P = 0.219$, Fig. 1). This was also reflected by non-significant associations of increased salivary tau with poorer global cognitive performance as assessed with MMSE ($r = -0.077$, $P = 0.198$) and CDR sum of boxes (median, 8.7 ng/L for CDR=0 and 10.2 ng/L CDR=$\leq$0.5; $P = 0.314$). There was no association between salivary tau concentration and measures of ventricular volume ($r = -0.048$, $P = 0.784$), hippocampal volume ($r = 0.068$, $P = 0.686$), entorhinal cortical thickness ($r = 0.088$, $P = 0.292$) and entorhinal cortex volume ($r = 0.107$, $P = 0.458$). However, when the AD group was analysed separately, a nominal association of lower salivary tau with greater ventricular volume was observed ($\rho = -0.492$, $P = 0.045$).

DISCUSSION – We observed no statistically significant difference in the levels of salivary tau across diagnostic groups. Additionally, we demonstrated that there was no association of salivary tau with MMSE and CDR sum of boxes, although the trends observed might indicate higher salivary tau with poorer cognition. In a subset, MRI measures were not associated with salivary tau. However, in the AD group alone, larger ventricle size was nominally associated with salivary tau.

The only other study reporting on salivary tau describe an increase in AD [7]. One explanation for these contradicting results is the difference in analytical methods used. In our study, the Simoa assay employed uses a combination of antibodies that react with both normal and phosphorylated tau with epitopes in the mid- and N-terminal regions of the molecule, making the assay specific for most tau isoforms. Shi *et al*. used a Luminex assay that measures the phosphorylated proportion (p-tau [181]) separately form t-tau and it was the p-tau/t-tau ratio that was increased in AD in their study. It is important to note that Shi *et al*. equally reported no difference in t-tau between aged-matched controls and AD.

At present, it is hard to imagine how sampling of saliva could produce results with a clear link to changes in the brain, given the many biological barriers and compartments the marker has to cross on its way to the sampling site. Furthermore, expression of, for example, tau mRNA and protein in

salivary glands and other extra-cerebral tissues such as the kidney (tau data in the Human Protein Atlas) could further limit the interpretability of measurements in saliva. Nevertheless, saliva has certain advantages over blood and CSF as a fluid for biomarker assessment. Its collection is less invasive and it is a minimally complex matrix that does not clot. Functions of the saliva are not only restricted to digestion. Saliva contains a large collection of proteins involved in the immune defence and the neuroendocrine system [10]. Further, it contains peptides that are in common with the CSF [10]. It is also possible that the innervation of the salivary gland could provide a more direct link between the saliva and the CNS than via the blood. The submandibular gland is responsible for the vast majority of total resting and stimulated salivary volume and has been reported to be dysfunctional in AD [11]. Therefore, saliva could be a rich source of novel biomarkers for AD [12] but there are major challenges in standardisation of collection and pre-processing methods ahead.

There are limitations to this study. Firstly, we are inferring that the AD patients in this study do indeed have increased tau pathology. Further clarification should seek to correlate salivary tau concentrations with CSF or PET measures. Similar studies investigating alpha-synuclein in Parkinson's disease demonstrate no relationship between saliva and CSF concentrations [13]. Secondly, the disproportionate numbers between the diagnostic groups could have potentially masked meaningful differences as we observe a non-significant median elevation in AD patients. In summary, total tau is reliably measured in human saliva using the Simoa platform and exists in a range of concentrations that are not systematically different between AD and non-AD diagnostic groups. We conclude that salivary tau is not a reliable biomarker for AD, nor a surrogate measure of cognition or brain atrophy.

REFERENCES

1. Palmqvist, S., et al., *Cerebrospinal fluid analysis detects cerebral amyloid-beta accumulation earlier than positron emission tomography.* Brain, 2016. **139**(Pt 4): p. 1226-36.
2. Samgard, K., et al., *Cerebrospinal fluid total tau as a marker of Alzheimer's disease intensity.* Int J Geriatr Psychiatry, 2010. **25**(4): p. 403-10.
3. Olsson, B., et al., *CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis.* Lancet Neurol, 2016. **15**(7): p. 673-684.

4. Zetterberg, H., et al., *Plasma tau levels in Alzheimer's disease.* Alzheimers Res Ther, 2013. **5**(2): p. 9.
5. Mattsson, N., et al., *Plasma tau in Alzheimer disease.* Neurology, 2016. **87**(17): p. 1827-1835.
6. Randall, J., et al., *Tau proteins in serum predict neurological outcome after hypoxic brain injury from cardiac arrest: results of a pilot study.* Resuscitation, 2013. **84**(3): p. 351-6.
7. Shi, M., et al., *Salivary tau species are potential biomarkers of Alzheimer's disease.* J Alzheimers Dis, 2011. **27**(2): p. 299-305.
8. Lovestone, S., et al., *AddNeuroMed--the European collaboration for the discovery of novel biomarkers for Alzheimer's disease.* Ann N Y Acad Sci, 2009. **1180**: p. 36-46.
9. Hye, A., et al., *Plasma proteins predict conversion to dementia from prodromal disease.* Alzheimers Dement, 2014. **10**(6): p. 799-807 e2.
10. Jasim, H., et al., *The proteomic profile of whole and glandular saliva in healthy pain-free subjects.* Sci Rep, 2016. **6**: p. 39073.
11. Ship, J.A., et al., *Diminished submandibular salivary flow in dementia of the Alzheimer type.* J Gerontol, 1990. **45**(2): p. M61-6.
12. Carro, E., et al., *Early diagnosis of mild cognitive impairment and Alzheimer's disease based on salivary lactoferrin.* Alzheimers Dement (Amst), 2017. **8**: p. 131-138.
13. Goldman, J.G., et al., *Cerebrospinal fluid, plasma, and saliva in the BioFIND study: Relationships among biomarkers and Parkinson's disease Features.* Mov Disord, 2018. **33**(2): p. 282-288.

Table 1. Summary of the demographic and clinical data of study participants

	Controls (n = 160)	**Mild Cognitive Impairment (n = 68)**	**Alzheimer's disease (n = 53)**	***P* value**
Age, y (s.d)	78.8 (6.7)	79.3 (7.4)	81.4 (6.6)	0.006
Sex, male/female (% female)	66/94 (758.7)	33/35 (51.5)	23/30 (56.6)	ns
Education, y (s.d)	13.9 (3.4)	12.1 (3.3)	11.7 (2.5)	>0.001
***APOE* genotype** (% ε4 carriers) {missing}	26 (32) {79}	9 (42.8) {47}	14 (58.3) {26}	0.041
MMSE (s.d)	28.9 (1.1)	26.8 (2.3)	22.3 (5.7)	>0.001
CDR [sum of boxes] (s.d, range)	0.15 (0.24, 0-0.5)	0.48 (0.14, 0-1)	0.89 (0.82, 0-3)	>0.001

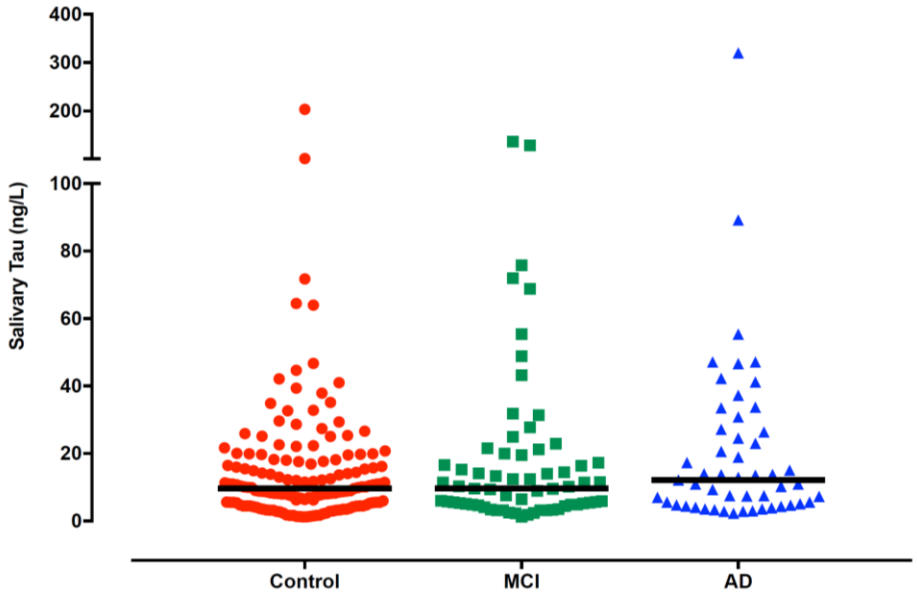

Figure 1. Salivary tau in controls, patients with mild cognitive impairment (MCI), and patients with Alzheimer disease (AD) dementia.

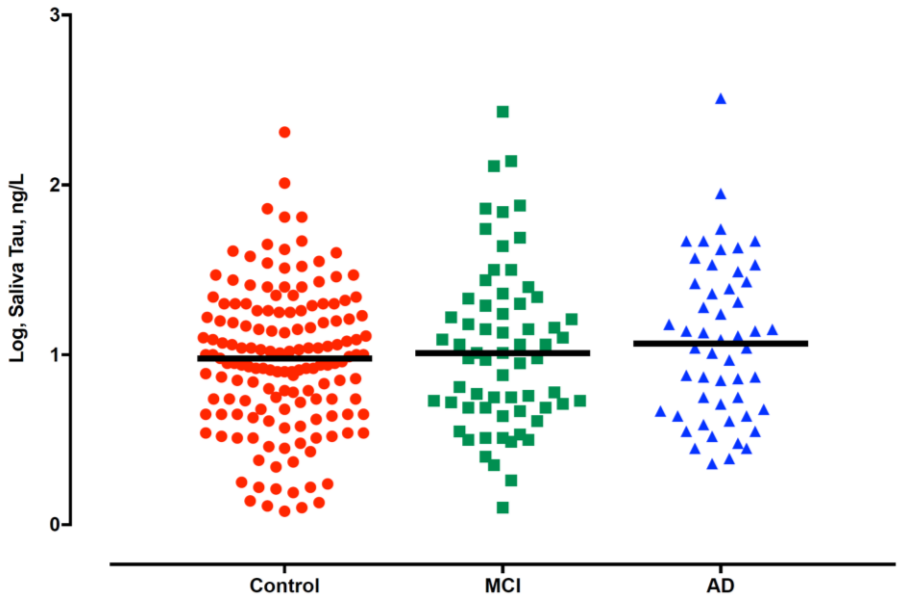

Figure 1. Salivary tau in controls, patients with mild cognitive impairment (MCI), and patients with Alzheimer disease (AD).