Diagnostic Assessment & Prognosis

Early diagnosis of mild cognitive impairment and Alzheimer's disease based on salivary lactoferrin

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Abstract

Introduction: The Alzheimer's disease (AD) process is likely initiated many years before clinical onset. Biomarkers of preclinical disease are critical for the development of disease-modifying or even preventative therapies. Current biomarkers for early disease, including cerebrospinal fluid tau and amyloid β (Aβ) levels, structural and functional magnetic resonance imaging, and the use of brain amyloid imaging, are limited because they are very invasive or expensive. Noninvasive biomarkers may be a more accessible alternative, but none can currently detect preclinical AD with the required sensitivity and specificity.

Methods: Here, we show a novel, straight-forward, and noninvasive approach for assessment of early stages of cognitive decline. Salivary samples from cases of amnestic mild cognitive impairment (aMCI) and AD, and neurology controls were analyzed.

Results: We have discovered and validated a new single saliva biomarker, lactoferrin, which in our cross-sectional investigation perfectly discriminates clinically diagnosed aMCI and AD patients from a cognitively healthy control group. The accuracy for AD diagnosis shown by salivary lactoferrin was greater than that obtained from core cerebrospinal fluid (CSF) biomarkers, including total tau and CSF Aβ$_{42}$. Furthermore, salivary lactoferrin can be used for population screening and for identifying those underdiagnosed subjects with very early stages of mild cognitive impairment and AD.

Conclusion: This biomarker may offer new insights in the early diagnostics for AD.

1. Background

Alzheimer's disease (AD) is the most common neurodegenerative disorder of the aging population, and because of the increase in longevity, the prevalence of AD is expected to raise dramatically. Because AD process is probably initiated many years before the clinical onset [1], biomarkers of preclinical disease are critical for the development of disease-modifying or even preventative therapies [2]. Unfortunately, current biomarkers for early disease, including cerebrospinal fluid tau and amyloid β (Aβ) levels [3], structural and functional magnetic resonance imaging [4], and the use of brain amyloid imaging or inflammaging [5,6], are limited because they are either invasive, time consuming, or expensive. Therefore, detecting AD at the earliest possible stage is vital to enable trials of disease modification agents and considerable efforts are being invested in the identification.

Saliva testing is currently used in areas of toxicology, endocrinology, infectious diseases, and forensics, with established diagnostic tests available for alcohol detection, HIV infections, hormonal analyses, and drug testing. Because saliva collection is noninvasive and relatively stress free, saliva can serve as a potential alternative and universal diagnostic fluid. Identification of Aβ and tau [7,8], or α-Syn and DJ-1 [9] in human saliva, proteins that are critically involved in AD and Parkinson's disease (PD), respectively, support the potential diagnostic value of saliva for neurodegenerative diseases.

A history of systemic infection is a known risk factor for AD [10–12]. Brain infections with bacteria or viruses are implicated in AD pathogenesis [13], but the impact of antimicrobial peptides on disease outcomes has not been sufficiently explored. Saliva is one of the body's first lines of defense due to its composition of antimicrobial proteins. Lactoferrin, one of the major antimicrobial peptides in saliva, represents an important defensive element by inducing a broad spectrum of antimicrobial effects against bacteria, fungi, protozoa, viruses, and yeasts [14–17], through its ability to decrease bacterial growth, biofilm development, iron overload, reactive oxygen formation, and regulating the inflammatory response [18,19].

The primary aim of our study was to investigate the potential of an AD diagnostic biomarker in saliva. We first carried out an AD diagnostic cross-sectional study and enrolled 274 participants at the Neurology Service at the Hospital Universitario 12 de Octubre (Madrid, Spain). We defined four groups of subjects according to their cognitive status: amnestic mild cognitive impairment (aMCI), AD, PD, and cognitively healthy control group. We discovered in this first diagnostic training study that saliva lactoferrin, an iron- but also Aβ-binding [20,21] glycoprotein, was strongly correlated with AD. We secondly validated the saliva lactoferrin as AD biomarker in two new blinded and independent cohorts. Finally, salivary levels of lactoferrin were examined in two independent longitudinal cohorts composed of healthy nondemented individuals.

2. Methods

2.1. Subjects and clinical classification

For the cross-sectional study, we included four groups of donors in the training study: (n = 80) AD patients; (n = 44) aMCI patients; (n = 59) PD patients; and (n = 91) elderly

Table 1
Demographic and clinical characteristics of subjects from first training study

Variable	Control	aMCI	AD	PD	P value
n (F/M)	91 (59/32)	44 (25/19)	80 (49/31)	59 (32/27)	ns
Age (years)	73.7 ± 6.88	75.16 ± 5.13	76.2 ± 5.33**	69.5 ± 8.6**	<.01
MMSE score	29 ± 0.8	26.8 ± 1.16***	19.25 ± 1.76***	NA	<.001
CDR score	0	0.5	≥1	NA	
APOE ε4 carriers	12.9%	42.1%**	45.9%**	NA	<.01
Education					
Can read and write	22.2%	36%	38.5%	NA	
Primary studies	33.3%	36%	33.3%		
Secondary studies	44.4%	28%*	28.2%*		<.05

Abbreviations: aMCI, amnestic mild cognitive impairment; AD, Alzheimer's disease; PD, Parkinson's disease; F, female; M, male; ns, not significant; MMSE, Mini–Mental State Examination; NA, not applicable; CDR, Clinical Dementia Rating.

NOTE. Data are expressed as mean ± SD. *$P < .05$ versus control group; **$P < .01$ versus control group; ***$P < .001$ versus control group.

nondemented controls, recruited from Neurology service of the University Hospital 12 de Octubre (Madrid, Spain) (Table 1). In the validation study, subjects from two independent entities, Pablo de Olavide University from Sevilla, Spain, and Alzheimer Disease Research Unit, CIEN Foundation, Queen Sofia Foundation Alzheimer Center (Madrid, Spain), were included and were divided into AD patients (n = 36), mild cognitive impairment (MCI) patients (n = 15), and elderly nondemented controls (n = 40) (Supplementary Table 1).

The AD diagnosis was established according to the National Institute on Neurological Disorders and Stroke, and the Alzheimer's Disease and Related Disorders Association guidelines [22]. MCI was diagnosed in patients with cognitive impairment according to the Petersen criteria [23]. Disease severity was evaluated using Mini–Mental State Examination (MMSE) scores. MCI patients had an MMSE of 26.8 ± 0.8; and AD patients had a MMSE of 19.25 ± 0.7. PD patients were diagnosed under the criteria of probable PD [24,25]. Inclusion criteria for cognitively normal older individual subjects were MMSE scores of 29 ± 0.8, no history or clinical signs of neurological or psychiatric disease or cognitive symptoms, as shown in Supplementary Table 2. Subjects' consent was obtained according to the Declaration of Helsinki, and approval was obtained from the Research Ethic Committee of each entity. Written informed consent was obtained from all participants or representatives.

For a 5-year longitudinal study, two independent and blinded cohorts were included in this study. In the first independent cohort, 116 healthy volunteers were enrolled from Neurology Service at the University Hospital 12 de Octubre. In a second independent cohort, 190 neurologically healthy individuals selected from sample donors at the Biobanco imas12 of the University Hospital 12 de Octubre were included as a confirmatory group. At the end of the year 5 of the study, some of these individuals met criteria for aMCI/AD.

Whole saliva was collected from patients with aMCI, AD, as well as healthy control subjects including samples from dementias other than AD (Table 1). Unstimulated whole saliva was collected into sterile plastic containers precoated with 2% sodium azide solution, as described previously [7]. Collected samples were immediately placed on ice and precleared by a low spin at 600× g for 10 minutes at 4°C. Aliquoted 0.5-mL samples were stored at −80°C after treatment with Protease Inhibitor Cocktail (Roche). Protein estimation was analyzed using a BCA protein assay kit (Pierce, Rockford, IL, USA) according to the manufacturer's instructions.

Oral mucosa was collected into sterile plastic containers according to the study by Aagaard et al. [26]. Briefly, 190 participants (M = 110/F = 80; average age = 62 ± 1.23) drooled into a 50-mL collection tube after allowing saliva to collect in the mouth for ≥1 minute, centrifuged at 6000× g for 10 minutes at 4°C, and pellets were stored at −80°C. Oral mucosa samples and united data from patients included in this study were provided by the Biobanco imas12 in the Hospital 12 de Octubre integrated in the Spanish Hospital Biobanks Network (RetBioH; www.redbiobancos.es) following standard operation procedures with appropriated approval of the Ethical and Scientific Committees.

Cerebrospinal fluid (CSF) samples (10 mL per subject) were obtained by lumbar puncture on informed patients consent. All samples were spun at 3000 rpm at 4°C for 10 minutes to remove any cells and debris, aliquoted in small volumes, and stored in low bind polypropylene tubes at −80°C.

2.2. Genotyping

Genomic DNA was extracted from peripheral blood using Illustra blood genomic Prep Mini Spin Kit (GE Healthcare). Apolipoprotein E (*APOE*) genotyping (2ε/3ε/4ε isoforms) was performed using FRET probes.

2.3. Mass spectrometry

Saliva samples from four male subjects from each group (MCI, AD, and elderly nondemented controls) were pooled by mixing equal amounts. Fifty micrograms of each pool were loaded on a SDS-PAGE gel. ImageQuant software (GE Healthcare) was used for quantity determination.

Protein bands matching the corresponding molecular weight were excised from the gel and distained. In-gel digestion was carried out sequentially with trypsin and endopeptidase Asp-N for 16 h each. To validate the presence of lactoferrin in human saliva, this protein was identified by MALDI-TOF/TOF mass spectrometer 4800 Proteomics Analyzer (Applied Biosystems, Framingham, MA) and 4000 Series ExplorerTM software (Applied Biosystems).

2.4. Biochemical analyses

Levels of lactoferrin in saliva samples were determined using the lactoferrin human ELISA kit (ab108882, Abcam) according to manufacturer's instructions. Levels of endogenous $A\beta_{42}$ and tau in CSF samples were determined using the $A\beta_{42}$ human ELISA Innotest kit (Innogenetics), and tau human ELISA Innotest kit (Innogenetics), respectively.

2.5. Statistical analysis

Qualitative variables are expressed with the percentage and the 95% confidence interval. Quantitative variables are expressed with the mean ± standard deviation (SD) in case they follow a normal distribution and with the median and interquartile range in case they follow nonnormal distribution. To determine the relation between salivary lactoferrin levels and the presence or not of AD, a multiple regression analysis was performed. To assess the association between levels of lactoferrin and the presence or absence of disease, crude linear regression model is constructed and adjusted for potentially confounding variables. To select
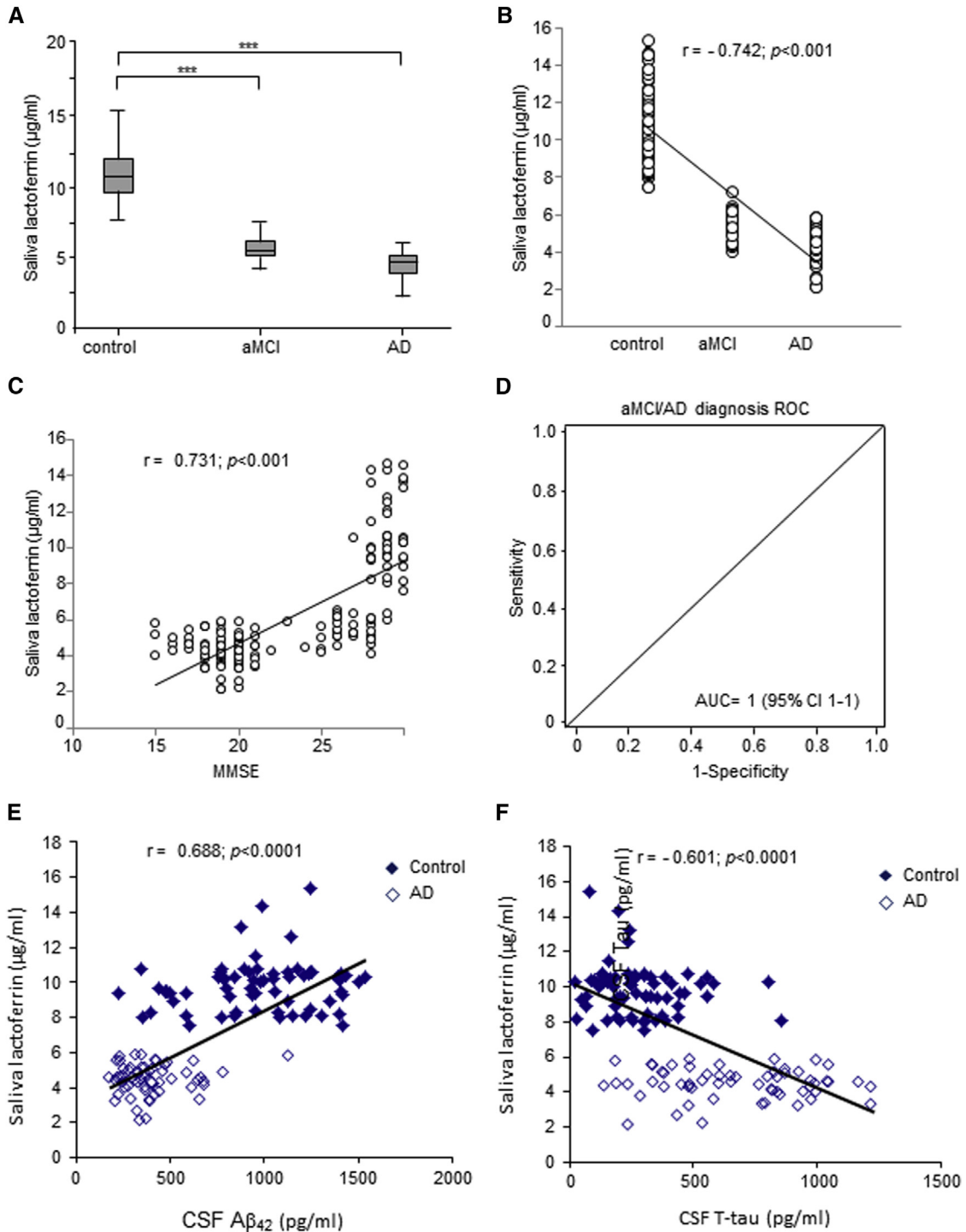
Fig. 1. Salivary lactoferrin levels in patients with aMCI, AD, and healthy controls. (A) Lactoferrin levels decrease in aMCI and AD compared with control group. Boxplot graph shows median, interquartile range, and extreme values of each group. ***$P < .001$; Kruskal-Wallis test. For lactoferrin expression in pooled saliva samples, see Supplementary Fig. 1. For additional data on lactoferrin levels in PD, see Supplementary Fig. 2. (B) Correlation between saliva levels of lactoferrin and cognitive decline in aMCI and AD groups. Lactoferrin levels appeared to be negatively correlated with severity of the disease (r = −0.742; $P < .001$; Kendall's tau correlation analysis). (C) Saliva levels of lactoferrin correlated with MMSE score, (r = 0.731; $P < .001$; Spearman's correlation analysis). (D) Receiver operating characteristic (ROC) curve obtained for the test of saliva lactoferrin levels from the full control group and aMCI/AD group. The area under the ROC curve (AUC), a measure of how well a parameter can distinguish between diagnostic groups, was 1 (95% CI 1–1). The ROC plots represent sensitivity (true positive rate) versus 1 − specificity (false positive rate). Salivary lactoferrin significantly correlates with $A\beta_{42}$ (E) and total tau (F) in CSF, based on Spearman's correlation analysis. Abbreviations: aMCI, amnestic mild cognitive impairment; AD, Alzheimer's disease; CSF, cerebrospinal fluid; MMSE, Mini–Mental State Examination; PD, Parkinson's disease; T-tau, total tau.

potential confounding variables to be included in the model, a bivariate analysis with each of them and the dependent variable (lactoferrin levels) was performed. We only selected statistically significant ($P < .05$) variables. The bivariate analysis included a Student's t-test (two-tailed) or Mann-Whitney U-test for single pairwise comparisons depending on variable distribution, and the Kruskal-Wallis test for multiple comparisons. Statistical significance was set at $P < .05$. The Spearman rank and Kendall's tau correlation was used for correlation analyses. In addition, receiver operating characteristic (ROC) curve analysis was used to determine cutoff points of salivary lactoferrin. The cutoff level for dichotomizing values was selected as the situation optimizing sensitivity and specificity. The classification performance of salivary lactoferrin was assessed using area under the ROC curve (AUC). The ROC can be understood as a plot of the probability of classifying correctly the positive samples against the rate of incorrectly classifying true negative samples. Thus, the AUC measure of an ROC plot is a measure of predictive accuracy. Youden index was calculated to select the optimum cutoff point. Data analysis was performed using the SPSS v20.0 software. A nominal P value of less than .05 was considered to indicate statistical significance. The statistical power of the study was 80%.

3. Results

Lactoferrin expression was first analyzed in pooled saliva samples from AD and aMCI patients, and control subjects, after SDS-PAGE fractionation and identification by mass spectrometry analysis (31% sequence coverage; Supplementary Fig. 1A). The band intensity analysis showed reduced lactoferrin levels in aMCI and AD compared with the control group (Supplementary Fig. 1B). Further confirmation by ELISA analysis in each individual donor sample revealed that salivary lactoferrin levels were significantly reduced in aMCI and AD patients compared with the healthy control group (Fig. 1A). A significant negative association was found between stages of disease (aMCI and AD) and salivary lactoferrin levels (Kendall's tau = -0.742; $P < .001$; Fig. 1B). Salivary lactoferrin concentration was also correlated with MMSE score in patients with aMCI and AD ($r = 0.731$; $P < .001$; Fig. 1C). Furthermore, we found that *APOE* ε4 allele status correlated with decreased lactoferrin levels in saliva (Pearson's = -0.204; $P < .001$).

Using linear regression analysis, we discovered that patients suffering from AD and aMCI had 6.432 μg (95% confidence interval [CI]: 6.850–6.014; $P < .001$) and 5.310 μg (95% CI: 5.810–4.810; $P < .001$) of salivary lactoferrin per milliliter less than cognitively healthy participants, respectively. We used these results to build linear classifier models that would distinguish the aMCI/AD groups from the control group, using ROC analysis. An AUC of 1 (95% CI 1–1) was obtained being the sensitivity 100% (95% CI 96.90%–100%) and specificity 100% (95% CI 95.95%–100%) for aMCI/AD and healthy control group

classification (Fig. 1D), and the cutoff value was 7.43 μg/mL (Youden index: 1).

To evaluate whether this reduction of lactoferrin was specific to AD, we measured lactoferrin concentration in saliva samples from 59 PD patients (Table 1). Salivary lactoferrin levels in PD patients were higher ($P < .001$) than those observed in the control group (Supplementary Fig. 2). These findings are in agreement with neuronal upregulation of lactoferrin in PD patients, as previously reported [27,28]. The linear regression model excluded patient's age as a potential confounding variable previously selected in the bivariate analysis. Additional analysis ruled out the potential influence of comorbidities, including diabetes, obesity, cardiovascular disease, and hypertension on salivary lactoferrin levels.

We then validated the cutoff value of saliva lactoferrin in two new blinded and independent cohorts enrolling 91 additional participants with the same standardized clinical assessments used in the previous study. Demographic characteristics of participants recruited in the Alzheimer Disease Research Unit, CIEN Foundation, Queen Sofia Foundation Alzheimer Center (Madrid, Spain), and Pablo de Olavide University (Sevilla, Spain) are shown in Supplementary Table 1. Results showed that the previously determined cutoff value of saliva lactoferrin (7.43 μg/mL) perfectly classified all patients (MCI/AD; $n = 51$) and all cognitively healthy subjects ($n = 40$).

The relationship between saliva lactoferrin and CSF total tau and CSF Aβ$_{42}$ levels was also addressed in a 127-subject subcohort (Table 2). Table 3 summarizes the correlation between the analytes in the control group and AD patients, based on Spearman's r correlation analysis. As shown in Table 3, and Fig. 1E and F, saliva lactoferrin significantly correlates with CSF Aβ$_{42}$ ($r = 0.688$, $P < .0001$; Fig. 1E), and CSF total tau ($r = -0.601$, $P < .0001$; Fig. 1F).

In our case-control studies, nondemented control subjects from clinical cohorts were subjected to strict inclusion and exclusion criteria. These clinical cohorts have been used in the training and validation studies as well as in the CSF

Table 2
Demographic, clinical, and biochemical characteristics of subjects from a subcohort study

Variable	Control	AD	P value
n (F/M)	68 (43/25)	59 (44/15)	ns
Age (years)	69.53 ± 8	80.07 ± 7.6***	<.001
APOE ε4 carriers	17.64%	49.15%**	<.01
CSF total tau (pg/mL)	250.71 ± 195.87	650.56 ± 469.71**	<.01
CSF Aβ$_{42}$ (pg/mL)	983.05 ± 461.83	366.97 ± 163***	<.001
Saliva lactoferrin (μg/mL)	10.24 ± 1.96	4.78 ± 1.11***	<.001

Abbreviations: AD, Alzheimer's disease; F, female; M, male; ns, not significant; CSF, cerebrospinal fluid.

NOTE. Age data are expressed as mean ± SD. Biomarkers data are expressed as median (interquartile range). **$P < .01$, ***$P < .001$ versus control group.

Table 3
Correlation matrix of saliva and CSF biomarkers in the control group versus AD patients

Correlations			
Biomarkers	Saliva lactoferrin	CSF Aβ₄₂	CSF total tau
Saliva lactoferrin	1	0.688***	−0.601***
CSF Aβ₄₂	0.688***	1	−0.529***
CSF total tau	−0.601***	−0.529***	1

Abbreviations: CSF, cerebrospinal fluid; AD, Alzheimer's disease.
NOTE. ***$P < .0001$.

biomarker correlation study. However, one should be cautious when generalizing from findings based on control samples recruited from clinic. Thus, we also decided to analyze salivary samples from "nonclinical" subject groups (with less detail available about the participants, but being more representative of the general population). We used two independent longitudinal cohorts composed of healthy nondemented individuals. A first cohort with 116 subjects, including caregivers, family, and other volunteers (Supplementary Table 3), was recruited between 2009 to 2014 at the Neurology Service, and the Group of Neurodegenerative Diseases at the Hospital Universitario 12 de Octubre, and one saliva sample [7] was collected. A second cohort of 190 apparently neurologically healthy subjects (Supplementary Table 4) was recruited at the Biobanco imas12 of the Hospital Universitario 12 de Octubre (Madrid, Spain), and oral mucosa [26] samples were collected at one time point from 2011 to 2012. The clinical status of all patients was evaluated in a follow-up examination (July 2015). Levels of lactoferrin in oral mucosa were equal to those measured in saliva samples. We found 18 subjects with abnormally reduced lactoferrin levels (<7.43 μg/mL) and 288 with normal/high lactoferrin levels (≥7.43 μg/mL). After checking their clinical diagnostic status, we de-

Table 4
Demographic characteristics of subjects from both longitudinal cohorts

Subjects	Gender	Age	Onset	Lt levels (μg/mL)	Neurological diagnose
1	M	82	2	3.01	aMCI
2	F	70	4	3.17	aMCI
3	F	71	5	3.69	aMCI
4	F	68	5	5.10	aMCI/AD
5	F	81	1	1.65	aMCI/AD
6	F	77	2	1.89	aMCI
7	M	83	3	6.18	aMCI/AD
8	M	88	4	4.45	aMCI
9	F	96	4	5.02	AD
10	M	66	4	3.66	aMCI
11	M	82	4	2.51	AD
12	M	67	4	5.92	aMCI
13	M	84	4	4.17	AD
14	M	85	3	4.13	aMCI/AD

Abbreviations: Lt, lactoferrin; M, male; aMCI, amnestic mild cognitive impairment; F, female; AD, Alzheimer's disease.

tected that over the course of the study (range 1–5 years, Table 4), 14 of 18 subjects had converted to a clinical diagnosis of aMCI or AD, whereas none of the subjects with a negative test value had converted to aMCI or AD. The ROC for the collectively analyzed data from both independent cohorts classified underdiagnosed patients and healthy control groups with an AUC of 0.984 (95% CI 0.932–1), with a sensitivity of 100%, and a specificity of 98.6% (Supplementary Fig. 3).

4. Discussion

Herein, we present the discovery and validation of salivary lactoferrin as a novel aMCI/AD diagnostic biomarker. According to our first study, salivary lactoferrin perfectly classified all aMCI/AD patients and all cognitively healthy subjects and it showed a very high correlation with validated CSF biomarkers. Furthermore, in our second study ("nonclinical" cohorts), we found apparently healthy individuals with low levels of saliva lactoferrin who were at high risk of converting to aMCI/AD dementia (more than 77%). As a consequence, and although more clinical studies are needed, we suggest that salivary lactoferrin is a precise and robust biomarker for aMCI/AD diagnosis and may help to identify, after a general population screening, those "apparently healthy" subjects that suffer from underdiagnosed later stage preclinical AD or even MCI.

It is fairly well recognized that clinical diagnosis of aMCI/AD patients is challenging [29,30]. However, accuracy of AD clinical diagnosis by the specialized physicians in this study is around 90%, similar to that reported elsewhere [31]. Interestingly, after a more indepth evaluation of the results obtained in the biomarker correlation study (salivary lactoferrin vs. CSF total tau and CSF Aβ₄₂), we found that although all cognitively normal participants had normal/high lactoferrin levels (≥7.3 μg/mL), 7 of 68 (∼10%) may have preclinical AD pathology, based on their CSF Aβ₄₂ levels. After monitoring the clinical evolution of these 7 subjects, we found that one of them, with lactoferrin levels of 8 μg/mL (close to the cutoff value), converted to MCI 6 years later. This may suggest that the decline of salivary lactoferrin happens in a later stage of the preclinical AD process, mainly when subtle cognitive deficit appears, taking into account the hypothetical model of the chronology [32].

In addition, we found that 7 of 59 (11.9%) patients clinically diagnosed with AD showed normal levels of CSF Aβ₄₂. Four of these 7 patients had normal CSF Aβ₄₂ levels but high CSF total tau levels. The latter may suggest that salivary lactoferrin may also work as a biomarker of cortical/cognitive dysfunction associated to other types of dementia in addition to AD. In fact, these 4 patients were clinically diagnosed with mixed AD dementia, including vascular component and dementia with Lewy bodies. Our results also provide evidence that salivary lactoferrin may work for identifying "apparently healthy" subjects that

suffer from later stage preclinical AD or aMCI, a high number of which are currently underdiagnosed.

Lactoferrin, an important modulator of immune response and inflammation [33], represents an important defensive element by inducing a broad spectrum of antimicrobial effects [14,16,17]. A novel role for antimicrobial peptides has been proposed in AD pathology as pathogen-targeting agents and markers of brain infections that are involved in the aggregation of amyloid [34], reinforcing the relationship between AD and brain infections [35].

To our knowledge, this is the first published report of a saliva-based single biomarker with very high accuracy for early diagnosis of AD. Saliva lactoferrin robustly classifies aMCI and AD patients from healthy control subjects. The accuracy for detection is equal to or greater than that obtained from other published blood and CSF studies [36,37]. However, saliva is by far more convenient and easier to obtain and costs less to acquire compared with blood and CSF. In addition, our biomarker consists of a single protein, lactoferrin, in contrast to others based on a set of proteins, lipids, or arrays of RNAs, making it more useful for screening in large-scale clinical trials and for future clinical use.

Further longitudinal cohort analyses are needed to address how salivary lactoferrin marker may help to differentiate between AD and other neurodegenerative diseases, including dementia with Lewy bodies or frontotemporal dementia. Finally, we plan to study the correlation of salivary lactoferrin levels with core CSF biomarkers, and PET neuroimaging, and potential confounding variables, including comorbid disorders, physiological status, or diet. Although these new studies are highly recommended, our initial study provides robust evidence for the capability of salivary lactoferrin to identify patients suffering from aMCI/AD. Moreover, it may also work for identifying "apparently healthy" subjects that suffer from later stage preclinical AD or aMCI, a high number of which are currently underdiagnosed. We believe our results may represent a significant advance in the National Institute on Aging and Alzheimer's Association consensus statement mandate for biomarkers of preclinical AD.

Acknowledgments

Authors' contributions: E.C. and G.O. conceived of the study. Experiments were coordinated and scientifically directed by E.C., and the article written by E.C. and G.O. Recruitment of patients and control individuals, and disease assessment in centres, was done by F.B., F.B.-P., A.V., J.A.M., P.O., M.C., A.R., and J.L.C.
The authors declare competing financial interests: E.C. and G.O. are founders of GEROA Diagnostics. Correspondence and requests for materials should be addressed to carroeva@h12o.es and gorka.orive@ehu.eus.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dadm.2017.04.002.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature using PubMed and reported key publications. Current biomarkers for early disease, including cerebrospinal fluid tau and amyloid β levels, structural and functional neuroimaging, are limited because they are very invasive or expensive. Noninvasive biomarkers may be a more accessible alternative, but none can currently detect preclinical Alzheimer's disease (AD) with the required sensitivity and specificity.

2. Interpretation: This study showed a novel, straightforward, and noninvasive approach for assessment of early stages of cognitive decline. We have discovered and validated a new single saliva biomarker, lactoferrin, which in our cross-sectional investigation perfectly discriminates clinically diagnosed amnestic mild cognitive impairment and AD patients from control group.

3. Future directions: Although future prospective studies will be highly recommended, this biomarker may represent a major turning point in the early diagnostics and decision making for patient care in AD.

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