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Plasma Tau Variants Detected by a Novel Anti-Tau Monoclonal Antibody: A Potential Biomarker for Alzheimer's Disease

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Abstract.
Background: A major drawback in Alzheimer's disease (AD) is the lack of validated biomarkers for routine clinical diagnostic. We have reported earlier a novel blood biomarker, named Alz-tau®, based on variants of platelet tau. This marker evaluates the ratio of high molecular weight tau (HMWtau) and the low molecular weight (LMWtau) tau.
Objective: To analyze a potential novel source of antigen for Alz-tau®, plasma tau, detected by immunoreactivity with the novel monoclonal antibody, tau51.
Methods: We evaluated tau variants in plasma precipitated with ammonium sulfate from 36 AD patients and 15 control subjects by western blot with this novel monoclonal antibody.
Results: The HMW/LMWtau ratio was statistically different between AD patients and controls.
Conclusions: Plasma tau variants are suitable to be considered as a novel antigen source for the Alz-tau® biomarker for AD.

Keywords: Alz-tau®, Alzheimer's disease, HMW/LMWtau ratio, monoclonal antibodies, peripheral biomarkers, tau protein in the human plasma

INTRODUCTION

Alzheimer's disease (AD) is a multifactorial, neurodegenerative pathology characterized by extracellular deposits of amyloid-β peptide (Aβ) in plaques, as well intracellular polymers such as paired helical filaments and neurofibrillary tangles resulting from tau oligomerization [1, 2]. Clinically, AD is characterized by a progressive cognitive impairment and behavioral disorders of patients, affecting around 12% of people >65 years [3]. AD is the main cause of dementia in the elderly (accounts for a 60–70% of cases) [4], thus affecting not only the patient, but also their caregivers. Consequently, considering the accelerated aging rate worldwide [5], this disease has turned in recent years in a serious public health issue.

Our neuroimmunomodulation theory [6] states that several danger signals (e.g., reactive oxygen species, Aβ oligomers, etc.) trigger microglial and astroglial activation, which leads to a neuroinflammatory microenvironment due to a chronic activation of the innate immune system, leading to neuronal dysfunction and finally neuronal death [6, 7].

Tau oligomers are constituted by hyperphosphorylated tau (p-tau), due to an excessive activity of CDK5 and GSK3β [8]. These oligomers lead to neurodegeneration, and due to that, tau aggregates are released to the extracellular domain, where they can act as danger signals to activate microglia and astrocytes, promoting the proinflammatory microenvironment

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[9]. Thus, paired helical filaments and neurofibrillary tangles are neurotoxic agents for the brain. The latter can also be applied to Aβ oligomers [10] which can exacerbate the inflammatory response.

Considering that AD is a multifactorial disease, and that current treatments only mitigate its symptoms [11, 12], early diagnosis is critical. Currently, there are no biomarkers that can diagnose AD in a preclinical or clinical stage. The only ones currently available are biomarkers in cerebrospinal fluid (CSF) [13, 14], positron emission tomography [15, 16], and magnetic resonance imaging [17], which are expensive and/or highly invasive [18]. Also, some of them require complementation with neuropsychology.

Recently, some potential blood-based biomarkers have been reported for preclinical diagnosis of AD, as well as to predict disease progression. The majority has focused on the Aβ peptide, which is a widely studied biomarker for AD [19]. However, due to the heterogeneity of the sources and contradictory results, further investigation is required. Another potential biomarker is CSF p-tau by electro-chemiluminescence using two monoclonal antibodies [20]. However, plasma p-tau is still under validation and even though if successful could be sensitive and specific, the cost due to the requirement of two monoclonal antibodies and required equipment, would made the implementation difficult at large scale. One of the few validated biomarkers involves tau protein evaluated in CSF [13]. Not only it has been correlated successfully with hippocampal volume [14], but also with cognitive decline [21]. However, it is highly invasive.

Recently, a novel non-invasive biomarker was developed, based on platelet tau which was detected by western blot [22, 23], and discriminates between AD patients and controls. This biomarker, named Alz-tau®, is based on an algorithm in which the ratio of two variants detected in platelets, high-molecular weight (HMW) and low-molecular weight (LMW) tau are evaluated by densitometry. Currently, Alz-tau® has been clinically validated by three clinical trials [24–26], and currently a fourth ongoing study is underway to validate the novel monoclonal antibody tau51. The marker using this antibody has a sensitivity of a 75.7% and a specificity of a 73.7% [25], which is appropriate for a novel technique.

In the present investigation, we present a reliable novel antigen source for the Alz-tau® biomarker, the plasma tau, consistent with our previous work, to support the Alz-tau® biomarker as an early predictor for AD.

METHODS

Subjects

The subjects for this study were recruited from the Ace Foundation, Barcelona, Spain, previously studied in Alz-tau® clinical trial [24]. For the purpose of the present study focused on plasma tau, we examined 36 probable AD subjects (Mini-Mental Status Exam (MMSE) score <28 and Global Deterioration Scale (GDS) >2) and 15 control subjects (MMSE score >28 and GDS < 2). The range of patients age in both groups of the study was between 52 to 91 years old (see Table 1). All the procedures were approved by the Medical Ethics Committee of the International Center for Biomedicine and Medical Ethics of the Ace Foundation, and all participants and/or their legal guardian signed the informed consents.

Plasma tau analyses

6 mL of venous blood samples were obtained from the patients and voluntary control subjects and subjected to differentiated centrifugation to separate the platelets. First the blood samples were centrifuged at 250 RCF (Relative Centrifuge Force) for 10 min at room temperature (RT), to obtain platelet-rich plasma. Next, plasma was centrifuged at 1750 RCF for 10 min at RT in order to obtain the isolated platelets. Platelets were then used for clinical trial of the Alz-tau® biomarker, and the plasma was stored at –20°C until use for this study.

Plasma was incubated with $(NH_4)_2SO_4$ at 45% (e.g., for 1.5 mL of plasma, 0.42 g of $(NH_4)_2SO_4$ was added) for 1 h at RT. Then, the mixture was centrifuged at 16000 RCF for 30 min. Pellet 1 (P1) was stored at –20°C and supernatant was incubated with ammonium sulfate at 60% for 1 h at RT (for 1 mL of supernatant, 0.1 g of $(NH_4)_2SO_4$ was added). Then, this new mixture is centrifuged at 16000 g for 30 min. Pellet 2 (P2) and supernatant (S) were stored at –20°C. Pellet 1 (the one which has tau) was resuspended in 500 μL of TBS 1× supplemented with protease inhibitors (Roche) and protein concentration was determined by nanodrop.

Production of the anti-tau monoclonal antibody (tau51)

The tau51 monoclonal antibody was produced according to monoclonal production technology [27], by injecting the highly purified platelet tau protein

Table 1

Demographic and clinical characteristics of the groups of Alzheimer's disease and healthy subjects (*). (From Guzmán-Martinez et al, 2019 [24])

	AD patients	Healthy subjects
Total subjects	36	15
Men	7	6
Women	29	9
GDS	3.97 ± 0.77	1.53 ± 0.52
CDR	1.11 ± 0.57	0.07 ± 0.18
MMSE	21.69 ± 3.90	29.47 ± 0.74
Age (mean)	74.92 ± 10.89 (52-91)	65.87 ± 6.63 (55-78)
Plasma ratio HMW/LMWtau**	0.4647 ± 0.0313	0.2982 ± 0.03670
Platelets ratio HMW/LMWtau***	1.445 ± 0.439	1.098 ± 0.349

*Plasma samples employed for this study were obtained from the same cohort as Guzmán-Martinez et al., 2019 [24]. **Ratio calculated from samples of human plasma obtained from blood according with Material and Methods. ***Platelets data was added for comparison purposes.

into Balb/c mice (0.2 mg/mouse) aged 6 to 7 weeks. After primary injection and a booster, B cells from the popliteal lymph nodes were fused with SP2/0-AG14 myeloma cells, in RPMI medium with 10% FBS, the resulting granule was collected and plated in cell culture plates. After culturing for 10 days in selection media with hypoxanthine, aminopterin, and thymidine (HAT), the hybridomas were screened and subcloned, to ensure that viable antibody-producing hybridomas are available after the selection process. The hybridomas were grown in RPMI 1640 + L-glutamine culture medium supplemented with 10% FBS, 1x Penicillin/streptomycin (100 I.U/mL), and 1 mM sodium pyruvate. Finally, antibodies were purified by a Protein A column, quantified and stored at –20°C. Hybridomas were produced on the basis of service purchase agreement, by Dr. Laura Bover of the Monoclonal Center at MD Anderson Hospital in Texas.

SDS-PAGE and western blot

50 μg of total protein from P1 1/10 were loaded in 10% polyacrylamide gels, and then transferred to a nitrocellulose membrane of 0.45 μm pore at 330 mA for 90 min. The membrane was blocked with 5% non-fat milk in 1X TBS for 1 h at RT. It will be used as specific primary antibody tau51 (1 : 1000), allowing to incubate in constant agitation overnight at 4°C. The membranes were incubated with the conjugated secondary antibody HRP 1 : 7000 (Pierce) for 1 h at RT. Immunoreactive bands were detected using appropriate reagents for chemiluminescence. The films were scanned, and the intensities of the bands quantified using Fiji for MAC OS and a ratio of tau of high molecular weight (HMWtau > 80 kDa) and of low molecular weight (LMWtau < 80 kDa) was calculated for each sample, both control subjects and AD patients.

Statistical analysis

The levels of the biomarker between AD and control were compared using a two-tailed Student's t-statistic for unrelated samples, using Graph Pad Prism program, version 5.0 for mac. The level of significance was set at 0.05 to consider the significant differences in the different statistical analyzes.

RESULTS

As similarly observed in previous studies regarding Alz-tau® and platelet tau as a marker [24], immunoblots presented an electrophoretic pattern immunoreactive with the monoclonal antibody tau51, with molecular weights ranging from approximately 55 kDa (the expected molecular weight for monomeric tau) to sizes as large as 230 kDa (approximately) in which each lane represents one patient (Fig. 1). The ratio of plasma tau was as much for healthy subjects as for AD, according to those described by Neumann et al. [23], with some modifications. As seen in Fig. 1, HMW immunoreactive bands (>70 kDa) for tau appears in both AD and healthy subjects, but more marked in AD. Based on Fig. 1, in Fig. 2 a 3D analysis was performed in order to highlight the differences among AD and Control. In this figure, it can be appreciated that HMW peaks are higher in AD patients than controls. However, it should be noted that the AD and control definition

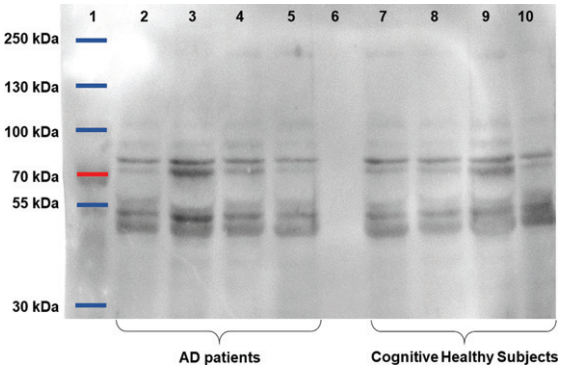

Fig. 1. Representative western blots of plasma tau with tau51 antibody. High molecular weight tau bands (≥80 kDa), showed increased immunoreactivity in AD patients compared to healthy subjects. On the other hand, the bands corresponding to low molecular weight tau (≤70 kDa), showed similar immunoreactivity in both control subjects and patients with AD. Lane 1: protein ladder; lanes 2-5: AD patients; lane 6: empty; lanes 7-10 Healthy controls. Each lane represents one patient.

is based on parameters of neuropsychology, thus it is possible that cognitive healthy subjects may have altered biomarker (Fig. 2).

According to the western blot densitometric analyzes of each subject under study, it was observed that the ratio HMW/LMWtau is significantly higher in the AD group in relation to the healthy subjects ($p = 0.033$) (Figs. 2 and 3A). Due to the fact that the HMW/LMW tau ratio is < 1, it can be sustained that the oligomeric form of tau (HMWtau) is found in less quantity than the low molecular weight variant (LMWtau) in plasma. The difference, however, relies that the levels of HMWtau in AD patients are significantly higher than in controls (see Figs. 1 and 2)

Figure 3B shows the ROC curve data for platelet tau ratio as a biomarker for AD. In this analysis, a cut-off point of 0.3897 for HMW/LMWtau ratio displayed a sensitivity of 80.00% and a specificity of 66.67% to discriminate AD and control subjects.

DISCUSSION

In this work, we describe a new tau biomarker for early detection of AD based on a novel source of tau antigen, in the human plasma. This is of relevance to generate an innovative a non-invasive peripheral

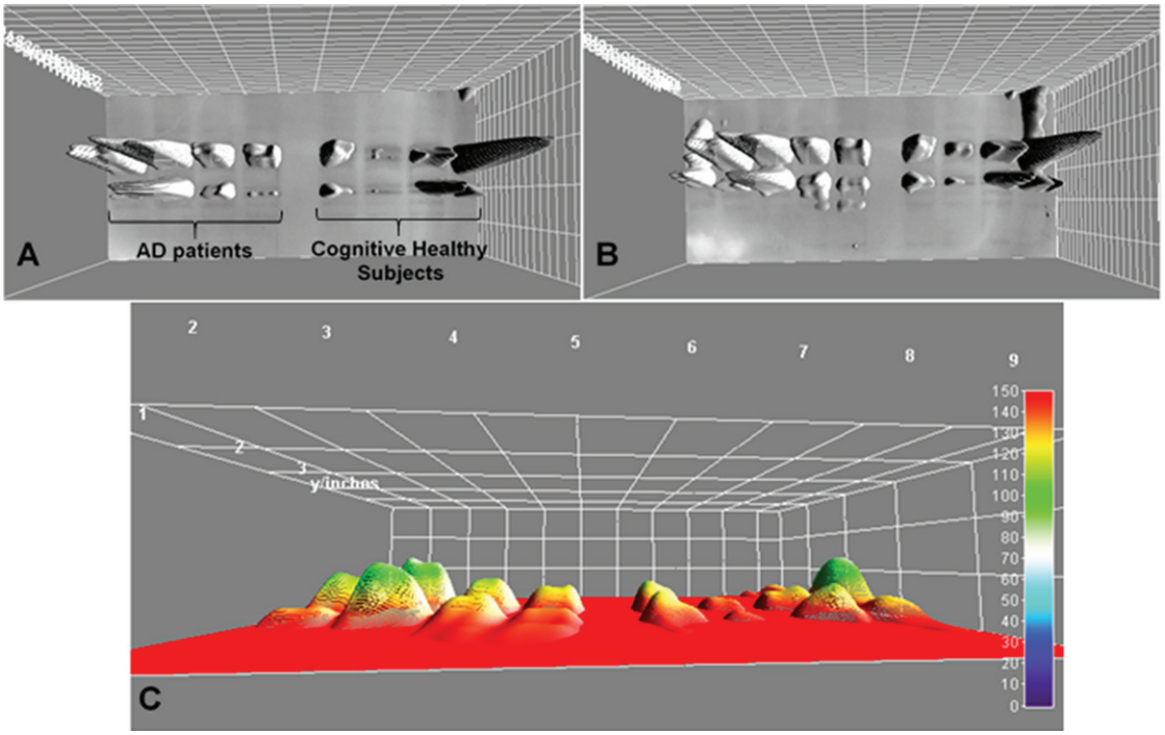

Fig. 2. Representative 3D immunoblotting of tau plasma with tau51 antibody. Image shows the difference in the intensity of the bands between AD patients and cognitively healthy subjects, according to the increase in exposure time (A) 20 seconds and (B) 40 seconds. C shows the highest density of the bands of the electrophoretic pattern in AD patients compared to the bands obtained in control subjects.

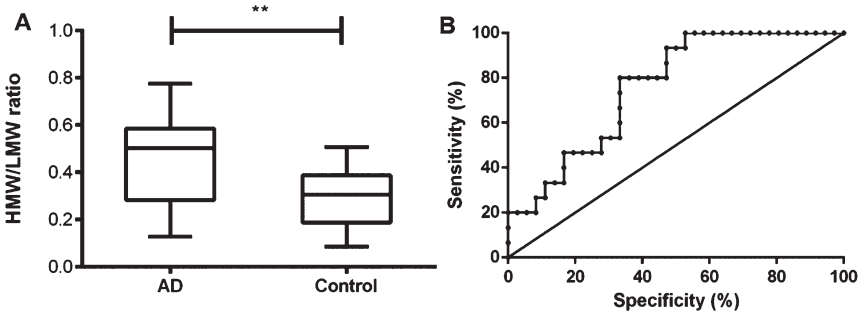

Fig. 3. HMW/LMW ratio is different when compared AD and Control. A) Graphic in which HMW/LMW ratio is represented in arbitrary units of densitometric quantization of tau species. This ratio is higher in AD patients when compared to controls. Two tailed T-student test, $p = 0.033$. Note that HMW/LMWtau values are less than 1, instead the Alz-tau® done in platelets has values higher than 1. B) Receiver-Operating Characteristic curve (ROC curve) of plasma tau, results obtained are similar to those obtained with platelet tau, appropriate to a novel technique. Area under the curve corresponds to 0.759. Sensibility of 80% and specificity of 66.5% (IC 95 : 0-6280 to 0.8905).

biomarker for AD. This potential biomarker derives from the already validated one, the Alz-tau® [22–25]. The difference between the present biomarker tool and our previously developed biomarker is that it is based in searching tau protein in plasma samples, as compared with the original Alz-tau® in which the target is the human platelet. Therefore, this human plasma approach appears as a useful and easier alternative procedure, in addition to the previously described Alz-tau® in human platelets, in order to have a reliable biomarker for diagnosis and early detection purposes. Firstly, this complementary approach has some advantages as compared with the platelet procedure: 1) The procedure to obtain the samples is easier, as it will not require platelet processing. 2) The plasma procedure would expand the implementation in more clinical laboratories of the hospitals and health centers, since it is technically easier. The use of both assays will certainly strengthen the diagnosis as it will have confirmation using two different samples (platelets and plasma).

Using plasma precipitated with ammonium sulfate, we defined that a similar electrophoretic pattern to the one obtained with platelet is observed when a novel monoclonal antibody (tau51) is employed. Interestingly, when HMW/LMWtau ratio is compared to the platelet tau, it was observed that HMW/LMW ratio is <1 in plasma (see Fig. 3A), and >1 in platelets [24], which could be explained by the lower levels of oligomeric tau in plasma when compared to platelets, as demonstrated in research conducted by Parck et al. (2019), in which they showed that tau protein is in a very low quantity in plasma (in order of picograms), since it is an intracellular protein [28]. However, the results are consistent with the fact that the ratio HMW/LMWtau is higher in AD patients when compared to controls. The ROC curve, sensitivity and specificity are acceptable for a novel method, and similar to the one obtained with platelets [24].

Plasma tau has been evaluated as a peripheral marker for tauopathies, including AD [28, 29]. However, since plasma tau is increased in other diseases, such as Down syndrome, its specificity should be taken into consideration [20, 30]. Other potential blood-based biomarkers include Aβ [31], p-tau and plasma tau [20] and micro-RNAs [32]. However, Aβ-based biomarkers in plasma has not been correlated with cognitive impairment [31] and not been evaluated with other neurodegenerative diseases. The evaluation of p-tau and plasma tau is promising, but since it is evaluated by a novel high-performance ELISA, with two antibodies, it is not likely that it can be implemented at a large scale [20]. micro-RNA is still under evaluation [32]. Previous studies with Alz-tau® demonstrated that the HMW/LMWtau ratio is specific for AD, as in other neurodegenerative diseases, such as Parkinson's disease and Lewy body dementia, there was no difference obtained between those and controls (unpublished data). Thus, it is likely that using the same algorithm used in platelet tau, now in plasma tau may maintain the high specificity for AD, though this should be evaluated later. It should be noted, that cognitive healthy subjects can present an altered biomarker, in platelets and now, in plasma. This allow us to propose that Alz-tau® employing plasma and platelets, can be used for preclinical diagnosis of AD.

Conclusion

Plasma tau can be a candidate to be an antigenic source to be evaluated as a derivative alternative for

the platelets Alz-tau® biomarker. Its electrophoretic pattern resembles the one obtained with platelet tau, and the results obtained are also consistent with the HMW/LMWtau ratio being higher in AD patients when compared to controls. This approach has some advantages as compared with platelets counterpart, and therefore it can provide an additional biomarker to complement the tools for early detection of AD based on tau variants.

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REFERENCES

[1] Nistico R, Pignatelli M, Piccinin S, Mercuri NB, Collingridge G (2012) Targeting synaptic dysfunction in Alzheimer's disease therapy. *Mol Neurobiol* **46**, 572-587.

[2] Holtzman DM, Morris JC, Goate AM (2011) Alzheimer's disease: the challenge of the second century. *Sci Transl Med* **3**, 77sr71.

[3] Hampel H, Schneider LS, Giacobini E, Kivipelto M, Sindi S, Dubois B, Broich K, Nistico R, Aisen PS, Lista S (2015) Advances in the therapy of Alzheimer's disease: targeting amyloid beta and tau and perspectives for the future. *Expert Rev Neurother* **15**, 83-105.

[4] Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA (2003) Alzheimer disease in the US population: prevalence estimates using the 2000 census. *Arch Neurol* **60**, 1119-1122.

[5] Martinez-Cue C, Rueda N (2020) Cellular senescence in neurodegenerative diseases. *Front Cell Neurosci* **14**, 16.

[6] Maccioni RB, Rojo LE, Fernandez JA, Kuljis RO (2009) The role of neuroimmunomodulation in Alzheimer's disease. *Ann N Y Acad Sci* **1153**, 240-246.

[7] Morales I, Farias G, Maccioni RB (2010) Neuroimmunomodulation in the pathogenesis of Alzheimer's disease. *Neuroimmunomodulation* **17**, 202-204.

[8] Jayapalan S, Natarajan J (2013) The role of CDK5 and GSK3B kinases in hyperphosphorylation of microtubule associated protein tau (MAPT) in Alzheimer's disease. *Bioinformation* **9**, 1023-1030.

[9] Morales I, Jimenez JM, Mancilla M, Maccioni RB (2013) Tau oligomers and fibrils induce activation of microglial cells. *J Alzheimers Dis* **37**, 849-856.

[10] Wang W, Hou TT, Jia LF, Wu QQ, Quan MN, Jia JP (2019) Toxic amyloid-beta oligomers induced self-replication in astrocytes triggering neuronal injury. *EBioMedicine* **42**, 174-187.

[11] Thancharoen O, Limwattananon C, Waleekhachonloet O, Rattanachotphanit T, Limwattananon P, Limpawattana P (2019) Ginkgo biloba extract (EGb761), cholinesterase inhibitors, and memantine for the treatment of mild-to-moderate Alzheimer's disease: a network meta-analysis. *Drugs Aging* **36**, 435-452.

[12] Atri A (2019) Current and future treatments in Alzheimer's disease. *Semin Neurol* **39**, 227-240.

[13] Ritchie C, Smailagic N, Noel-Storr AH, Ukoumunne O, Ladds EC, Martin S (2017) CSF tau and the CSF tau/ABeta ratio for the diagnosis of Alzheimer's disease dementia and other dementias in people with mild cognitive impairment (MCI). *Cochrane Database Syst Rev* **3**, CD010803.

[14] de Souza LC, Chupin M, Lamari F, Jardel C, Leclercq D, Colliot O, Lehericy S, Dubois B, Sarazin M (2012) CSF tau markers are correlated with hippocampal volume in Alzheimer's disease. *Neurobiol Aging* **33**, 1253-1257.

[15] Zhang S, Smailagic N, Hyde C, Noel-Storr AH, Takwoingi Y, McShane R, Feng J (2014) (11)C-PIB-PET for the early diagnosis of Alzheimer's disease dementia and other dementias in people with mild cognitive impairment (MCI). *Cochrane Database Syst Rev*, CD010386.

[16] Martinez G, Vernooij RW, Fuentes Padilla P, Zamora J, Flicker L, Bonfill Cosp X (2017) 18F PET with florbetaben for the early diagnosis of Alzheimer's disease dementia and other dementias in people with mild cognitive impairment (MCI). *Cochrane Database Syst Rev* **11**, CD012883.

[17] Schmand B, Huizenga HM, van Gool WA (2010) Meta-analysis of CSF and MRI biomarkers for detecting preclinical Alzheimer's disease. *Psychol Med* **40**, 135-145.

[18] Tan CC, Yu JT, Tan L (2014) Biomarkers for preclinical Alzheimer's disease. *J Alzheimers Dis* **42**, 1051-1069.

[19] Koyama A, Okereke OI, Yang T, Blacker D, Selkoe DJ, Grodstein F (2012) Plasma amyloid-beta as a predictor of dementia and cognitive decline: a systematic review and meta-analysis. *Arch Neurol* **69**, 824-831.

[20] Tatebe H, Kasai T, Ohmichi T, Kishi Y, Kakeya T, Waragai M, Kondo M, Allsop D, Tokuda T (2017) Quantification of plasma phosphorylated tau to use as a biomarker for brain Alzheimer pathology: pilot case-control studies including patients with Alzheimer's disease and down syndrome. *Mol Neurodegener* **12**, 63.

[21] Kandimalla R, Manczak M, Yin X, Wang R, Reddy PH (2018) Hippocampal phosphorylated tau induced cognitive decline, dendritic spine loss and mitochondrial abnormalities in a mouse model of Alzheimer's disease. *Hum Mol Genet* **27**, 30-40.

[22] Guzman-Martinez L, Farias GA, Maccioni RB (2013) Tau oligomers as potential targets for Alzheimer's diagnosis and novel drugs. *Front Neurol* **4**, 167.

[23] Neumann K, Farias G, Slachevsky A, Perez P, Maccioni RB (2011) Human platelets tau: a potential peripheral marker for Alzheimer's disease. *J Alzheimers Dis* **25**, 103-109.

[24] Guzman-Martinez L, Tapia JP, Farias GA, Gonzalez A, Estrella M, Maccioni RB (2019) The Alz-tau biomarker for Alzheimer's disease: study in a Caucasian population. *J Alzheimers Dis* **67**, 1181-1186.

[25] Farias G, Perez P, Slachevsky A, Maccioni RB (2012) Platelet tau pattern correlates with cognitive status in Alzheimer's disease. *J Alzheimers Dis* **31**, 65-69.

[26] Slachevsky A, Guzman-Martinez L, Delgado C, Reyes P, Farias GA, Munoz-Neira C, Bravo E, Farias M, Flores P, Garrido C, Becker JT, Lopez OL, Maccioni RB (2017) Tau platelets correlate with regional brain atrophy in patients with Alzheimer's disease. *J Alzheimers Dis* **55**, 1595-1603.

[27] Milstein C (1982) Monoclonal antibodies. *Cancer* **49**, 1953-1957.

[28] Park JC, Han SH, Yi D, Byun MS, Lee JH, Jang S, Ko K, Jeon SY, Lee YS, Kim YK, Lee DY, Mook-Jung I (2019)

Plasma tau/amyloid-beta1-42 ratio predicts brain tau deposition and neurodegeneration in Alzheimer's disease. *Brain* **142**, 771-786.

[29] Mattsson N, Zetterberg H, Janelidze S, Insel PS, Andreasson U, Stomrud E, Palmqvist S, Baker D, Tan Hehir CA, Jeromin A, Hanlon D, Song L, Shaw LM, Trojanowski JQ, Weiner MW, Hansson O, Blennow K, ADNI Investigators (2016) Plasma tau in Alzheimer disease. *Neurology* **87**, 1827-1835.

[30] Startin CM, Ashton NJ, Hamburg S, Hithersay R, Wiseman FK, Mok KY, Hardy J, Lleo A, Lovestone S, Parnetti L, Zetterberg H, Hye A, LonDown SC, Strydom A (2019) Plasma biomarkers for amyloid, tau, and cytokines in Down syndrome and sporadic Alzheimer's disease. *Alzheimers Res Ther* **11**, 26.

[31] Nabers A, Perna L, Lange J, Mons U, Schartner J, Guldenhaupt J, Saum KU, Janelidze S, Holleczek B, Rujescu D, Hansson O, Gerwert K, Brenner H (2018) Amyloid blood biomarker detects Alzheimer's disease. *EMBO Mol Med* **10**.

[32] Kumar S, Reddy PH (2016) Are circulating microRNAs peripheral biomarkers for Alzheimer's disease? *Biochim Biophys Acta* **1862**, 1617-1627.