



**HAL**  
open science

## Plasma $A\beta_{42}$ as biomarker of prodromal AD progression in patients with amnesic mild cognitive impairment: evidence from the PharmaCog/E-ADNI study

Diego Albani, Moira Marizzoni, Clarissa Ferrari, Federica Fusco, Lucia Boeri, Ilaria Raimondi, Jorge Jovicich, Claudio Babiloni, Andrea Soricelli, Roberta Lizio, et al.

### ► To cite this version:

Diego Albani, Moira Marizzoni, Clarissa Ferrari, Federica Fusco, Lucia Boeri, et al.. Plasma  $A\beta_{42}$  as biomarker of prodromal AD progression in patients with amnesic mild cognitive impairment: evidence from the PharmaCog/E-ADNI study. *Journal of Alzheimer's Disease*, 2019, 69 (1), pp.37-48. 10.3233/JAD-180321 . hal-03575161

**HAL Id: hal-03575161**

**<https://hal.science/hal-03575161v1>**

Submitted on 15 Feb 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# **Plasma A $\beta$ 42 as biomarker of prodromal AD progression in patients with amnesic mild cognitive impairment: evidence from the PharmaCog/E-ADNI study**

Diego Albani<sup>1</sup>, Moira Marizzoni<sup>2</sup>, Clarissa Ferrari<sup>3</sup>, Federica Fusco<sup>1</sup>, Lucia Boeri<sup>1</sup>, Ilaria Raimondi<sup>1</sup>, Jorge Jovicich<sup>4</sup>, Claudio Babiloni<sup>5,6</sup>, Andrea Soricelli<sup>7</sup>, Roberta Lizio<sup>5</sup>, Samantha Galluzzi<sup>2</sup>, Libera Cavaliere<sup>2</sup>, Mira Didic<sup>8,9</sup>, Peter Schönknecht<sup>10</sup>, José Luis Molinuevo<sup>11</sup>, Flavio Nobili<sup>12</sup>, Lucilla Parnetti<sup>13</sup>, Pierre Payoux<sup>14</sup>, Luisella Bocchio<sup>15</sup>, Marco Salvatore<sup>7</sup>, Paolo Maria Rossini<sup>16,17</sup>, Magda Tsolaki<sup>18</sup>, Pieter Jelle Visser<sup>19</sup>, Jill C. Richardson<sup>20</sup>, Jens Wiltfang<sup>21,22,23</sup>, Régis Bordet<sup>24</sup>, Olivier Blin<sup>25</sup>, Gianluigi Forloni<sup>1</sup>, Giovanni B. Frisoni<sup>2,26</sup> and PharmaCog Consortium.

<sup>1</sup> Department of Neuroscience, IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy;

<sup>2</sup> Laboratory of Neuroimaging and Alzheimer's Epidemiology, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy;

<sup>3</sup> Unit of Statistics, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy;

<sup>4</sup> MR Lab Head, Center for Mind/Brain Sciences, University of Trento, Italy;

<sup>5</sup> Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, Rome, Italy;

<sup>6</sup> Department of Neuroscience, IRCCS San Raffaele Pisana of Rome and Cassino, Rome and Cassino, Italy;

<sup>7</sup> IRCCS SDN Istituto di Ricerca Diagnostica e Nucleare, Napoli, Italy;

<sup>8</sup> Aix-Marseille Université, INSERM, INS UMR\_S 1106, 13005, Marseille, France;

<sup>9</sup> APHM, Timone, Service de Neurologie et Neuropsychologie, APHM Hôpital Timone Adultes, Marseille, France;

<sup>10</sup> Department of Psychiatry and Psychotherapy, University of Leipzig, Leipzig, Germany, Germany;

<sup>11</sup> Alzheimer's Disease Unit and Other Cognitive Disorders Unit, Hospital Clínic de Barcelona, and Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Catalunya, Spain;

<sup>12</sup> Clinical Neurology, Dept. of Neuroscience (DINOEMI), University of Genoa and IRCCS AOU San Martino-IST, Genoa, Italy;

<sup>13</sup> Clinica Neurologica, Università di Perugia, Ospedale Santa Maria della Misericordia, Perugia, Italy;

<sup>14</sup> INSERM; Imagerie cérébrale et handicaps neurologiques UMR 825; F-31059 Toulouse, France;

<sup>15</sup> Genetic Unit, IRCCS Centro Giovanni di Dio, Fatebenefratelli, Brescia, Italy; Faculty of Psychology, University eCampus, Novedrate (Como), Italy;

<sup>16</sup> Department of Gerontology, Neurosciences & Orthopedics, Catholic University, Rome, Italy;

<sup>17</sup> Policlinic A. Gemelli Foundation;

<sup>18</sup> 3rd Neurologic Clinic, Medical School, G. Papanikolaou Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece;

<sup>19</sup> Department of Neurology, Alzheimer Centre, VU Medical Centre, Amsterdam, The Netherlands;

<sup>20</sup>Neurosciences Therapeutic Area, GlaxoSmithKline R&D, Gunnels Wood Road, Stevenage, United Kingdom;

<sup>21</sup>LVR-Hospital Essen, Department of Psychiatry and Psychotherapy, Faculty of Medicine, University of Duisburg-Essen, Essen, Germany;

<sup>22</sup> Department of Psychiatry and Psychotherapy, University Medical Center (UMG), Georg-August-University, Goettingen, Germany;

<sup>23</sup> iBiMED, Medical Sciences Department, University of Aveiro, Aveiro, Portugal;

<sup>24</sup> University of Lille, Inserm, CHU Lille, U1171 - Degenerative and vascular cognitive disorders, F-59000 Lille, France;

<sup>25</sup> Aix Marseille University, UMR-CNRS 7289, Service de Pharmacologie Clinique, AP-HM, Marseille, France;

<sup>26</sup> Memory Clinic and LANVIE - Laboratory of Neuroimaging of Aging, University Hospitals and University of Geneva, Geneva, Switzerland.

**Keywords:** Prodromal Alzheimer's disease; amnesic mild cognitive impairment (aMCI); Amyloid-beta peptide; Clusterin; Clinical trial; Biomarkers; PharmaCog project.

**Corresponding Author:**

Dr. Diego Albani

Neuroscience Department, IRCCS – Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy

Phone: +39-02-39014594

Fax: +39-02-35462770461-28-3066

Email: [diego.albani@marionegri.it](mailto:diego.albani@marionegri.it)

## ABSTRACT

It is an open issue whether blood biomarkers serve to diagnose Alzheimer's disease (AD) or monitor its progression over time from prodromal stages. Here, we addressed this question starting from data of the European FP7 IMI-PharmaCog/E-ADNI longitudinal study in amnesic mild cognitive impairment (aMCI) patients including biological, clinical, neuropsychological (e.g. ADAS-Cog13), neuroimaging and electroencephalographic measures. PharmaCog/E-ADNI patients were classified as "positive" (i.e., "prodromal AD"; N=76) or "negative" (N=52) based on a diagnostic cut-off of A $\beta$ 42/P-tau in cerebrospinal fluid as well as APOE $\epsilon$ 4 genotype. Blood was sampled at baseline and at two follow-ups (12 and 18 months), when plasma amyloid peptide 42 and 40 (A $\beta$ 42, A $\beta$ 40) and apolipoprotein J (clusterin, CLU) were assessed. Linear Mixed Models found no significant differences in plasma molecules between the "positive" (i.e., prodromal AD) and "negative" groups at baseline. In contrast, plasma A $\beta$ 42 showed a greater reduction over time in the prodromal AD than the "negative" aMCI group (p=0.048), while CLU and A $\beta$ 40 increased but similarly in the two groups. Furthermore, plasma A $\beta$ 42 correlated with the ADAS-Cog13 score both in aMCI patients as a whole and the prodromal AD group alone. Finally, CLU correlated with the ADAS-Cog13 only in the whole aMCI group, and no association with ADAS-Cog13 was found for A $\beta$ 40. In conclusion, plasma A $\beta$ 42 showed disease progression-related features in aMCI patients with prodromal AD.

## 1. INTRODUCTION

A current hot-spot of clinical research in Alzheimer's disease (AD) deals with the discovery of sensitive, specific, non-invasive, and cost-effective biomarkers useful for the diagnosis or the quantification of illness progression from prodromal stage (amnesic mild cognitive impairment, aMCI) to dementia stage, featuring severe cognitive deficits and disability in self-care and autonomy [1]. According to the current guidelines, as reported in Dubois et al. [1], diagnostic biomarkers of AD include low concentration of A $\beta$ 42 and high concentration of total tau (T-tau) or phospho-tau (P-tau) in cerebrospinal fluid (CSF), or evidence of significant amyloid deposition and tau aggregation in the brain in maps of positron emission tomography (PET). On the other hand, topographic or progression biomarkers of AD measure atrophy of hippocampus or cerebral cortex, as quantified in structural magnetic resonance imaging (MRI), and hypometabolism in posterior cingulate, parietal, temporal, and hippocampal regions, as measured by FDG-positron emission tomography (FDG-PET) [1]. Of note, the use of those procedures in AD clinical practice is relatively limited by invasiveness of the protocols or high-cost of instruments and exams.

The discovery of reliable blood biomarkers of AD would be a great improvement, as they are minimally invasive, potentially accessible everywhere, and intrinsically cost-effective. State-of-the-art in the field has been recently reviewed [2,3]. Many different biological targets have been proposed as blood biomarkers of prodromal AD, as those based on the amyloid precursor protein (A $\beta$ PP) processing, the molecules related to tangle pathology coming from tau dysregulation, markers of neurodegeneration and microglia/astocyte activation as neurofilament light (NF-L), neurogranin (Ng), sTREM2 and YKL-40, or AD-associated protein accumulation (for instance,  $\alpha$ -synuclein and TDP-43), up to microRNA (miRNA) quantification [2-7]. Unfortunately, literature results are contradictory, probably because of a lack of standardization in assays and clinical inclusion criteria. In particular, many studies were centered on the comparison of healthy controls and AD patients, a choice that might be a confounding factor for diagnostic or prognostic purpose [8-13].

Clusterin (apolipoprotein J, CLU) has also been suggested as candidate plasma biomarker of AD, based on CLU gene involvement in AD risk and the availability of several association studies assessing CSF or plasma CLU level in prodromal dementia [14-18].

Keeping in mind the above scenario, it is critical to underscore that some differences in blood biomarkers between AD patients and age-matched healthy controls with normal cognition may be unspecific for disease neuropathology. In other words, those biomarkers might be sensitive not only to AD but also to other disorders inducing cognitive deficits in seniors. To account for this confounding variable, here we took advantage from the prospective, multi-centric clinical study named “IMI-PharmaCog-European ADNI” ([www.pharmacog.org](http://www.pharmacog.org)), where 144 aMCI patients were followed over time with the collection of clinical, neuropsychological, structural and functional MRI, electroencephalographic (rsEEG/ERP), CSF, and blood data. In the present study, we specifically tested the hypothesis that blood plasma measured molecules A $\beta$ 42, A $\beta$ 40 and CLU may be able to diagnose AD and monitor its progression (i.e. a period of 18 months) from prodromal disease stages.

This article is part of a Mini Forum of Journal of Alzheimer’s disease on PharmaCog/E-ADNI matrix of biomarkers of prodromal AD in patients with aMCI.

## **2. MATERIALS AND METHODS**

### ***2.1. Participant clinical features and classification***

Participants’ demographics, clinical and neuropsychological data have been described in recent PharmaCog/E-ADNI studies. Briefly, 147 amnesic mild cognitive impairment (aMCI) patients were enrolled in 13 European memory clinics of the Innovative Medicine Initiative (IMI) PharmaCog/E-ADNI project ([www.pharmacog.org](http://www.pharmacog.org)). The protocol of this study was designed in the framework of IMI and was aimed at improving the pathway of drug discovery in AD, with a main interest on disease-modifying drugs reducing A $\beta$ 42 in the brain in AD patients at the prodromal stage of aMCI. Inclusion criteria were age between 55 and 90 years; complaints of memory loss; Mini-Mental State Examination (MMSE) score of  $\geq 24$ ; Clinical Dementia Rating score of 0.5; score on the logical memory test  $< 1$  standard deviation from the age-adjusted mean; 15-item Geriatric Depression Scale score  $\leq 5$  and no neurologic, systemic or psychiatric comorbidity [19-20]. We applied the diagnostic criteria for AD suggested by IWG-2 [1] and AA-NIH [21] guidelines. According to these guidelines, even at prodromal stage, AD is associated with (1) a reduction of cerebrospinal (CSF) A $\beta$ 42 and its increase at brain level and with (2) an increase of phospho-tau in both CSF and brain. IWG-2 and AA-NIH guidelines state that the diagnosis of AD can be done with A $\beta$ 42 and tau biomarkers even with a single recording session, as AD is considered a progressive disease

[1, 21]. Before study enrollment, each patient gave signed informed consent in compliance to the guidelines of local ethical committees. Data collected and generated have been always used in anonymous and aggregated form.

The aMCI patients were classified into two groups named “positive” (i.e., prodromal AD) and “negative” (i.e. stable aMCI) based on baseline CSF A $\beta$ 42/P-tau levels as well as APOE $\epsilon$ 4 genotype [22]. Specifically, aMCI patients were considered “positive” with CSF A $\beta$ 42/P-tau levels lower than 15.2 for APOE $\epsilon$ 4 carriers and lower than 8.9 for APOE $\epsilon$ 4 non-carriers, otherwise “negative”. These cut-offs were obtained by applying model-based classification methods (mixture models) [23] on baseline CSF A $\beta$ 42/P-tau distribution, adjusted for APOE $\epsilon$ 4 genotype.

## ***2.2. Blood collection and plasma separation***

All procedures involving patients were done after eligibility check according to inclusion criteria and informed consent signature. Blood for plasma preparation was collected by venipuncture at baseline, at month 12 and 18 during follow-up, resulting in a total of 3 venipuncture sessions.

Procedures for blood withdrawal and processing were standardized for all centers. Blood samples were processed within 1 hour from the puncture. Briefly, 10 mL of blood were collected into EDTA tubes of and centrifuged at 1600g/4°C/15min. The supernatant (plasma) was transferred into a new polypropylene tube after gentle shaking to avoid gradient effects and divided into aliquots of 250  $\mu$ L in dry ice. Plasma was kept frozen at -80°C in temperature-monitored ultra-freezers (-80°C  $\pm$  5°C) until required.

## ***2.3. Amyloid peptides 40 and 42 (A $\beta$ 40, 42) and clusterin (CLU) ELISA determination***

The assessment of plasma A $\beta$ 42 and 40 was done with ELISA kits from Fujirebio (Fujirebio, Japan), namely Innostest  $\beta$ -amyloid(1-42) (code 81576), in presence of high-sensitivity secondary antibody conjugate (code 81587), and Innostest  $\beta$ -amyloid(1-40) (code 81585). The limit of detection (LOD) for the kits were 4.0 and 5.0 pg/mL, respectively. The assays dynamic ranges were 7.8-1000 pg/mL and 6.8-1000 pg/mL, respectively.

Human clusterin (apolipoprotein J) concentration in plasma was measured by an ELISA kit (BioVendor – Laboratorní medicína a.s., Czech Republic, code BV53031). The kit limit of detection (LOD), defined as concentration of analyte giving absorbance higher than

mean absorbance of blank plus three standard deviations of the absorbance of blank, was 0.5 ng/ml. The assay dynamic range was from 5 to 160 ng/mL.

## ***2.4 Statistical analysis***

Statistics was done by SPSS software for descriptive statistics and R software (version 3.4.1) for the computational analysis based on Linear Mixed Models. The aMCI participants' features were compared by parametric Student's t-tests or non-parametric Mann-Whitney's U-test, depending on Gaussian distribution and using Chi-square tests for categorical data. Due to the exploratory nature of the present study, significance level was set at  $p < 0.05$  [24].

Two different types of Linear Mixed Models (LMMs, performed by R-package lme4) for repeated measures were used with all available values of the plasma biomarkers ( $A\beta$  42, 40, 42/40 and CLU) and clinical variables. Random intercept and random slope were considered to account for individual differences at baseline as well as for individual change over follow-up. The output of the LMMs was presented in terms of standardized  $\beta$  coefficient, corresponding p-value and effect size (pseudo  $\eta^2$ ) calculated as ratio of explained variability of interaction effect on total variability of each model.

In details, a first group of LMMs was conducted to identify plasma measured molecules (dependent variable) that differently progressed in prodromal AD compared to stable aMCI patients in the whole aMCI group. This was performed by adding age, gender, education, time, group (corresponding to CSF status), time X group interaction as covariates. Only plasma measures with significant group X time interaction were of interest, meaning that they differently progressed over-time between groups. A second group of LMMs was conducted to evaluate the association between cognitive changes (ADAS-Cog 13, dependent variable) and peripheral plasma measured molecules, in the whole group and in prodromal AD patients only. This was performed by adding age, gender, time and biomarker as covariates. Plasma assessed molecules showing a significant effect of the biomarker factor were of interest, meaning that they were associated to cognitive decline.

## **3. RESULTS**

### ***3.1. Patients' features***

In the IMI-PharmaCog/E-ADNI study, a cohort of 144 aMCI out of the 147 enrolled patients underwent CSF standard dementia biomarker evaluation ( $A\beta$ 42, T-tau, P-tau) and



apolipoprotein E (APOE) genotyping. **Table 1** summarizes IMI-PharmaCog/E-ADNI cohort demographic and clinical features. Due to plasma unavailability of some patients, the number of aMCI patients who were included for plasma plasma measure assessment was lower (i.e., 128 aMCI patients). The main demographic and clinical characteristics of the included patients are reported in **Table 2**. In both **Table 1** and **2**, after stratification according to baseline A $\beta$ 42/P-tau ratio values in the CSF as a function of APOE genotype [22], the aMCI patients were classified as “positive” (prodromal AD) or “negative”. We also statistically compared mean values reported in **Table 2** to **Table 1** in order to exclude a selection bias due to the unavailable samples in the plasma analysis. There were no differences between the “positive” (prodromal AD) and “negative” aMCI groups (data not shown).

### **3.2. Amyloid peptides 40 and 42 (A $\beta$ 40, 42), clusterin (CLU) and prodromal AD**

**Figures 1** to **4** summarize the results of an exploratory statistical analysis about plasma A $\beta$ 42, A $\beta$ 40, A $\beta$ 42/A $\beta$ 40 ratio, and CLU in the “positive” (prodromal AD) and “negative” aMCI groups at the three recording timepoints (T0, T12 and T18 months). The figures also show the same plasma measures in aMCI patients as a whole group. Exploratory univariate statistical tests compared the mean values between the groups or between timepoints ( $p < 0.05$ ).

**Figure 1** shows the results for plasma A $\beta$ 42. There was no significant mean difference between the two aMCI groups at any time ( $p > 0.05$ ). Furthermore, there was no significant mean difference among the three timepoints when all aMCI patients were considered as a whole group ( $p > 0.05$ ).

**Figure 2** plots the results for plasma A $\beta$ 40. There was a marginal significance when comparing T0 level between the two aMCI groups ( $p = 0.06$ ), with mean values slightly lower in the “positive” than the “negative” group. Furthermore, there was no significant mean difference among the three timepoints when all aMCI patients were considered together ( $p > 0.05$ ).

**Figure 3** illustrates the results for plasma A $\beta$ 42/A $\beta$ 40 ratio. There was no significant mean difference between the two aMCI groups at any time ( $p > 0.05$ ). Moreover, there was no difference among the three timepoints when all aMCI patients were grouped.

Finally, **Figure 4** describes the results for CLU. There was no significant mean difference between the two aMCI groups at any time ( $p > 0.05$ ). In contrast, CLU increased in all aMCI patients as a whole group over time, with a significant difference from T0 to both

T12 and T18 ( $p < 0.001$ ). This difference was common to the “negative” and “positive aMCI groups.

To refine the above statistical analysis, we applied Linear Mixed Models to the plasma measures using the factors Group (“positive” and “negative” aMCI) and Time (T0, T12, and T18). **Table 3** reports the proportion of variability in plasma measures over time explained by Time, Group (CSF status as defined by A $\beta$ 42/P-tau), and Time X Group interaction. All plasma measures considered reported a significant effect of Time (for A $\beta$ 42,  $p < 0.001$ ; A $\beta$ 40,  $p = 0.009$ ; A $\beta$ 42/A $\beta$ 40 ratio,  $p = 0.006$ ; CLU,  $p < 0.001$ ), showing their changes over time (T0 to T18) regardless the group. Conversely, none of those measures showed a significant “diagnostic” Group effect ( $p > 0.05$ ).

Noteworthy, there was a significant Time X Group effect for plasma A $\beta$ 42, showing that compared to the “negative” aMCI group, the “positive” (prodromal AD) aMCI group was characterized by a significant decrease of the measure over time ( $p < 0.05$ ), in line with the feature of a disease progression biomarker.

### ***3.4. Correlation of A $\beta$ 40, A $\beta$ 42, A $\beta$ 42/A $\beta$ 40, and clusterin (CLU) with ADAS-cog13 score***

**Table 4** reports the results of Linear Mixed Models testing the correlation over time of plasma measured molecules (A $\beta$ 42, A $\beta$ 40, A $\beta$ 42/A $\beta$ 40 ratio, and CLU) with ADAS-Cog13 score. When all aMCI patients were considered as a whole, there was a significant association with ADAS-Cog13 score for all plasma measures ( $p < 0.003$ ) with the only exception of A $\beta$ 40. This association reflected the increase of ADAS-Cog13 scores over the follow-up period due to a progressive cognitive impairment of the whole population.

When the “positive” (prodromal AD) aMCI group was considered alone, there was still a significant association between plasma A $\beta$ 42 ( $p < 0.05$ ) and ADAS-Cog13 score, thus suggesting a clinical relevance of that measure. The same was true for plasma A $\beta$ 42/A $\beta$ 40 ratio ( $p < 0.05$ ). Instead, no association was found for A $\beta$ 40 alone or CLU ( $p > 0.05$ ).

## **4. DISCUSSION**

The IMI-PharmaCog/E-ADNI longitudinal study aimed at testing candidate biomarkers suitable to diagnose prodromal AD in aMCI patients and track disease progression over time (up to 24 months). As a novelty in the field of biomarker discovery for aMCI progressing to

AD, to overcome the possible confounding effect of comparing healthy subjects to cognitively impaired patients, we used a control group with the same kind of amnesic deficits of the experimental group. Specifically, we compared blood plasma biomarkers in aMCI patients “positive” (i.e., prodromal AD) vs. “negative” classified basing on their CSF A $\beta$ 42/P-tau level and APOE $\epsilon$ 4 carrier status [22]. In the present investigation, we tested the diagnostic or disease monitoring value of plasma A $\beta$ 42, A $\beta$ 40, and CLU in aMCI patients with probable prodromal AD. Among many other plasma biomarker candidates, the present ones have obvious links to AD pathogenic mechanisms and a direct counterpart on relevant CSF and PET diagnostic measures used in AD research.

However, the collected plasma and DNA samples may be suitable for other AD blood biomarker candidates of interest, including a variety of protein, lipid, and microRNA species, as well as mitochondrial genes or DNA epigenetic modification patterns [25-32]. They may be evaluated in future studies carried out in PharmaCog/E-ADNI “positive” and “negative” aMCI groups.

Concerning the diagnostic value of the assessed blood biomarker candidates, the present results showed that plasma A $\beta$ 42 was not specifically associated with the group of “positive” aMCI patients (prodromal AD) when the three recordings (baseline, 12 and 18 months) were considered as a whole. Furthermore, plasma A $\beta$ 42, A $\beta$ 42/A $\beta$ 40 ratio, and CLU in all aMCI patients as a whole were correlated with cognitive status as measured by ADAS-Cog13 score, namely the neuropsychological procedure typically used in AD clinical trials [33, 34]. These findings suggest that plasma A $\beta$ 42, A $\beta$ 42/A $\beta$ 40 ratio, and CLU are clinically relevant for aMCI cognitive status and may partially explain the variance of the results in previous studies where plasma A $\beta$ 42 and A $\beta$ 40 (or their ratio) were informative on AD status, especially when AD patients with dementia were compared to seniors with intact cognition [8]. Indeed, this association between plasma biomarkers and AD status was not always confirmed [11, 13]. So large variance of results in previous investigations might partially depend on cognitive status of participants in the AD and control groups as well as disease stage of AD participants. Of course, technical reasons may also contribute to the observed variance in previous findings [35, 36]. For example, the importance of plasma A $\beta$ 42 as a biomarker of AD has been recently re-evaluated thanks to the contribution of Nakamura and colleagues, who measured plasma A $\beta$ 42 with an advanced high-performance procedure based on immunoprecipitation followed by mass spectrometry [7]. In light of this improved protocol, they were able to demonstrate an interesting correlation between plasma

A $\beta$ 42 measurements and CSF and PET biomarker counterparts in AD patients [7]. In addition, Nabers and colleagues developed an immune-infrared sensor to measure the secondary structure change of all soluble A $\beta$  peptides in human plasma that correlated to CSF AD biomarkers and amyloid PET in a cross-sectional study and was predictive of AD in a prospective cohort [37].

Concerning the informative value of the present plasma biomarkers on prodromal AD progression, the present results show that plasma A $\beta$ 42 was specifically associated with the “positive” aMCI group (prodromal AD) as a function of time (i.e., follow-ups at 12 and 18 months). The prodromal AD patients showed a specific significant decrease of plasma A $\beta$ 42 over time, which correlated with the deterioration of cognitive performance as revealed by ADAS-cog13 scores. To our knowledge, this is the first demonstration that plasma A $\beta$ 42 may be used as a biomarker of prodromal AD progression, taking into account the confounding variable of aMCI patients’ cognitive status.

Available literature shows mixed results about the possible correlation between CSF and plasma A $\beta$ 42. In our study, we checked for this correlation in “positive” aMCI subjects, finding no evidence (data not shown). Indeed, some previous studies failed in demonstrating a significant relationship [38, 39] while other were successful in finding a correlation, either positive [8], or negative [40]. Here we report that compared with the “negative” aMCI subjects, the “positive” aMCI showed a steeper longitudinal lowering in the A $\beta$ 42 at plasma level (interaction between Group x Time factors) but not a lowering considering all recording sessions as a whole (i.e., no Group factor effect). This outcome cannot be explained by an effect of different cognitive deficits in the experimental (“positive” MCI) and control (“negative” MCI) groups, as both were MCI (indeed, the condition of MCI might theoretically be due non only to AD neuropathology but also other parallel causes affecting cognitive functions, namely a cerebrovascular disease). A conclusive explanation of the above results requires further investigation. We can just speculate that plasma A $\beta$ 42 may be influenced not only by the brain amyloidosis but also by the interaction between such process and others related to AD (e.g., tauopathy and neurodegeneration). However, any interpretation of the results should take into account that our study focused on a limited time of follow-up (i.e., until 18-24 months) and that CSF could be collected only at baseline and after 18 months. Therefore, our findings are a proof-of-concept to be cross-validated with a longitudinal study in which A $\beta$ 42 in the CSF and plasma are systematically recorded in positive MCI subjects over time.

The second plasma biomarker investigated in the present study was clusterin (apolipoprotein J, CLU), based on the promising literature addressing the role of CLU in blood-based early AD diagnosis. In fact, it was reported that CLU levels are elevated in brain, cerebrospinal fluid (CSF), and plasma of AD patients with dementia and MCI [41]. Moreover, CLU is functionally associated with amyloid species, and many genetic association studies have confirmed its role as a predisposing factor for AD [42-45]. Despite these considerations, we were unable to show a significant value of CLU neither in prodromal AD diagnosis nor in the disease progression. There was, however, a slight increase of plasma CLU over time both in “negative” and “positive” aMCI groups, suggesting that this blood biomarker may track the progression of brain disorders but not specifically for AD. It can be speculated that this blood biomarker may have a slower variation with disease onset and progression in comparison to plasma A $\beta$ 42, and increased amyloid burden may be required to reveal robust CLU differential expression in brain or in the periphery. In the present experimental design, the plasma follow-up time (18 months) may be too limited to conclusively demonstrate an AD-specific variation of CLU longitudinally.

In conclusion, we suggest that after the diagnosis of aMCI according to criteria based on CSF A $\beta$ 42 lowering and p-tau increase [1, 21], also plasma A $\beta$ 42 measured with standard ELISA procedure may be sensitive to prodromal AD progression and cognitive impairment. Instead, we did not confirm a diagnostic value of plasma A $\beta$ 42 at least at that prodromal stage. We are confident that in a short-term period other studies may cross-validate our results, also taking advantage from recent technological advancements in the assessment of plasma A $\beta$ 42 [7], and we propose to speed-up plasma A $\beta$ 42 assay translation to clinical setting. Finally, our results on plasma A $\beta$ 42 may be integrated by future studies that systematically investigate the relationship between CSF vs. plasma phospho-tau and total tau, considering the remarkable steps forward in the measurement of those biomarkers [46].

## 5. ACKNOWLEDGMENTS

The IMI-PharmaCog/E-ADNI project was funded by the European seventh framework program and European Federation of Pharmaceutical Industries and Associations (EFPIA) for the Innovative Medicine Initiative (Grant no. 115009; [www.pharmacog.org](http://www.pharmacog.org)).

In 2017, G.F. and C.B. were partially supported by H2020 ITN-ETN MSCA project “BBDiag” (Grant no. 721281; <http://bbdiag-itn-etn.eu/>).

The authors are grateful to all members and collaborators and to aMCI patients who agreed to participate in this study. We thank Judith Baggott for English editing.

## 6. REFERENCES

- [1] Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, DeKosky ST, Gauthier S, Selkoe D, Bateman R, Cappa S, Crutch S, Engelborghs S, Frisoni GB, Fox NC, Galasko D, Habert MO, Jicha GA, Nordberg A, Pasquier F, Rabinovici G, Robert P, Rowe C, Salloway S, Sarazin M, Epelbaum S, de Souza LC, Vellas B, Visser PJ, Schneider L, Stern Y, Scheltens P, Cummings JL (2014) Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet Neurol* **13**, 614–629.
- [2] Zetterberg H, Blennow K (2018) From Cerebrospinal Fluid to Blood: The Third Wave of Fluid Biomarkers for Alzheimer's Disease. *J Alzheimers Dis* . doi: 10.3233/JAD-179926;
- [3] Shi L, Baird AL, Westwood S, Hye A, Dobson R, Thambisetty M, Lovestone S (2018) A Decade of Blood Biomarkers for Alzheimer's Disease Research: An Evolving Field, Improving Study Designs, and the Challenge of Replication. *J Alzheimers Dis* **62**,1181-1198.
- [4] Ovod V, Ramsey KN, Mawuenyega KG, Bollinger JG, Hicks T, Schneider T, Sullivan M, Paumier K, Holtzman DM, Morris JC, Benzinger T, Fagan AM, Patterson BW, Bateman RJ (2017) Amyloid  $\beta$  concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. *Alzheimers Dement* **13**, 841-849.
- [5] Delvaux E, Mastroeni D, Nolz J, Chow N, Sabbagh M, Caselli RJ, Reiman EM, Marshall FJ, Coleman PD (2017) Multivariate analyses of peripheral blood leukocyte transcripts distinguish Alzheimer's, Parkinson's, control, and those at risk for developing Alzheimer's. *Neurobiol Aging* **58**, 225-237.
- [6] Kumar S, Vijayan M, Reddy PH (2017) MicroRNA-455-3p as a potential peripheral biomarker for Alzheimer's disease. *Hum Mol Genet* **1**, 3808-3822.
- [7] Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Doré V, Fowler C, Li QX, Martins R, Rowe C, Tomita T, Matsuzaki K, Ishii K, Ishii K, Arahata Y, Iwamoto S, Ito K, Tanaka K, Masters CL, Yanagisawa K (2018) High performance plasma amyloid- $\beta$  biomarkers for Alzheimer's disease. *Nature* **554**, 249-254.
- [8] Hanon O, Vidal JS, Lehmann S, Bombois S, Allinquant B, Tréluyer JM, Gelé P, Delmaire C, Blanc F, Mangin JF, Buée L, Touchon J, Hugon J, Vellas B, Galbrun E, Benetos A, Berrut G, Paillaud E, Wallon D, Castelnovo G, Volpe-Gillot L, Paccalin M, Robert PH, Godefroy O, Dantoine T, Camus V, Belmin J, Vandel P, Novella JL, Duron E, Rigaud AS, Schraen-Maschke S, Gabelle A; BALTAZAR study group (2018) Plasma amyloid levels within the Alzheimer's process and correlations with central biomarkers. *Alzheimers Dement* pii: S1552-5260(18)30021-9.

- [9] Fandos N, Pérez-Grijalba V, Pesini P, Olmos S, Bossa M, Villemagne VL, Doecke J, Fowler C, Masters CL, Sarasa M; AIBL Research Group (2017) Plasma amyloid  $\beta$  42/40 ratios as biomarkers for amyloid  $\beta$  cerebral deposition in cognitively normal individuals. *Alzheimers Dement (Amst)* **8**:179-187.
- [10] Lövheim H, Elgh F, Johansson A, Zetterberg H, Blennow K, Hallmans G, Eriksson S (2017) Plasma concentrations of free amyloid  $\beta$  cannot predict the development of Alzheimer's disease. *Alzheimers Dement* **13**, 778-782.
- [11] Janelidze S, Stomrud E, Palmqvist S, Zetterberg H, van Westen D, Jeromin A, Song L, Hanlon D, Tan Hehir CA, Baker D, Blennow K, Hansson O (2016) Plasma  $\beta$ -amyloid in Alzheimer's disease and vascular disease. *Sci Rep* **6**, 26801.
- [12] Ritchie C, Smailagic N, Noel-Storr AH, Takwoingi Y, Flicker L, Mason SE, McShane R (2014) Plasma and cerebrospinal fluid amyloid beta for the diagnosis of Alzheimer's disease dementia and other dementias in people with mild cognitive impairment (MCI). *Cochrane Database Syst Rev* **6**:CD008782. doi: 10.1002/14651858.CD008782.pub4.
- [13] Toledo JB, Shaw LM, Trojanowski JQ (2013) Plasma amyloid beta measurements - a desired but elusive Alzheimer's disease biomarker. *Alzheimers Res Ther* **5**, 8.
- [14] Elias-Sonnenschein LS, Helisalmi S, Natunen T, Hall A, Paajanen T, Herukka SK, Laitinen M, Remes AM, Koivisto AM, Mattila KM, Lehtimäki T, Verhey FR, Visser PJ, Soininen H, Hiltunen M (2013) Genetic loci associated with Alzheimer's disease and cerebrospinal fluid biomarkers in a Finnish case-control cohort. *PLoS One* **8**:e59676.
- [15] Schrijvers EM, Koudstaal PJ, Hofman A, Breteler MM (2011) Plasma clusterin and the risk of Alzheimer disease. *JAMA* **305**, 1322-1326.
- [16] Jongbloed W, van Dijk KD, Mulder SD, van de Berg WD, Blankenstein MA, van der Flier W, Veerhuis R (2015) Clusterin Levels in Plasma Predict Cognitive Decline and Progression to Alzheimer's Disease. *J Alzheimers Dis* **46**, 1103-1110.
- [17] Gupta VB, Doecke JD, Hone E, Pedrini S, Laws SM, Thambisetty M, Bush AI, Rowe CC, Villemagne VL, Ames D, Masters CL, Macaulay SL, Rembach A, Rainey-Smith SR, Martins RN; AIBL Research Group (2015) Plasma apolipoprotein J as a potential biomarker for Alzheimer's disease: Australian Imaging, Biomarkers and Lifestyle study of aging. *Alzheimers Dement (Amst)* **3**, 18-26.
- [18] Haight T, Bryan RN, Meirelles O, Tracy R, Fornage M, Richard M, Nasrallah I, Yaffe K, Jacobs DR Jr, Lewis C, Schreiner P, Sidney S, Davatzikos C, Launer LJ (2018)



Associations of plasma clusterin and Alzheimer's disease-related MRI markers in adults at mid-life: The CARDIA Brain MRI sub-study. *PLoS One* **13**:e0190478.

[19] Galluzzi S, Marizzoni M, Babiloni C, Albani D, Antelmi L, Bagnoli C, Bartres-Faz D, Cordone S, Didic M, Farotti L, Fiedler U, Forloni G, Girtler N, Hensch T, Jovicich J, Leeuwis A, Marra C, Molinuevo JL, Nobili F, Pariente J, Parnetti L, Payoux P, Del Percio C, Ranjeva JP, Rolandi E, Rossini PM, Schönknecht P, Soricelli A, Tsolaki M, Visser PJ, Wiltfang J, Richardson JC, Bordet R, Blin O, Frisoni GB; PharmaCog Consortium (2016) Clinical and biomarker profiling of prodromal Alzheimer's disease in workpackage 5 of the Innovative Medicines Initiative PharmaCog project: a 'European ADNI study'. *J Intern Med* **279**, 576-591.

[20] Nathan PJ, Lim YY, Abbott R, Galluzzi S, Marizzoni M, Babiloni C, Albani D, Bartres-Faz D, Didic M, Farotti L, Parnetti L, Salvadori N, Müller BW, Forloni G, Girtler N, Hensch T, Jovicich J, Leeuwis A, Marra C, Molinuevo JL, Nobili F, Pariente J, Payoux P, Ranjeva JP, Rolandi E, Rossini PM, Schönknecht P, Soricelli A, Tsolaki M, Visser PJ, Wiltfang J, Richardson JC, Bordet R, Blin O, Frisoni GB; PharmaCog Consortium (2017) Association between CSF biomarkers, hippocampal volume and cognitive function in patients with amnesic mild cognitive impairment (MCI). *Neurobiol Aging* **53**,1-10.

[21] Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R; Contributors (2018) NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement* **14**,535-562.

[22] Marizzoni M, Ferrari C, Galluzzi S, Jovicich J, Albani D, Babiloni C, Didic M, Forloni G, Molinuevo JL, Nobili FM, Parnetti L, Payoux P, Rossini PM, Schönknecht P, Soricelli A, Tsolaki M, Visser PJ, Wiltfang J, Bordet R, Cavaliere L, Richardson J, Blin O, Frisoni GB (2017) CSF biomarkers and effect of apolipoprotein E genotype, age and sex on cut-off derivation in mild cognitive impairment. *Alzheimer's Dement* **13**, P1319.

[23] Geoffrey McLachlan, David Peel (2000) Finite Mixture Models. John Wiley & Sons, Inc. New York. ISBN: 978-0-471-00626-8.

[24] Bender R, Lange S (2001) Adjusting for multiple testing-when and how? *J Clin Epidemiol* **54**,343-349.

[25] Varma VR, Oommen AM, Varma S, Casanova R, An Y, Andrews RM, O'Brien R, Pletnikova O, Troncoso JC, Toledo J, Baillie R, Arnold M, Kastenmueller G, Nho K, Doraiswamy PM, Saykin AJ, Kaddurah-Daouk R, Legido-Quigley C, Thambisetty M (2018) Brain and blood metabolite signatures of pathology and progression in Alzheimer disease: A targeted metabolomics study. *PLoS Med* **15**:e1002482.

- [26] Wang MJ, Yi S, Han JY, Park SY, Jang JW, Chun IK, Kim SE, Lee BS, Kim GJ, Yu JS, Lim K, Kang SM, Park YH, Youn YC, An SSA, Kim S (2017) Oligomeric forms of amyloid- $\beta$  protein in plasma as a potential blood-based biomarker for Alzheimer's disease. *Alzheimers Res Ther* **9**, 98.
- [27] Reumiller CM, Schmidt GJ, Dhrami I, Umlauf E, Rappold E, Zellner M (2017) Gender-related increase of tropomyosin-1 abundance in platelets of Alzheimer's disease and mild cognitive impairment patients. *J Proteomics* pii: S1874-3919(17)30441-4.
- [28] Pedrini S, Gupta VB, Hone E, Doecke J, O'Bryant S, James I, Bush AI, Rowe CC, Villemagne VL, Ames D, Masters CL, Martins RN; AIBL Research Group (2017) A blood-based biomarker panel indicates IL-10 and IL-12/23p40 are jointly associated as predictors of  $\beta$ -amyloid load in an AD cohort. *Sci Rep* **7**, 14057.
- [29] Lunnon K, Keohane A, Pidsley R, Newhouse S, Riddoch-Contreras J, Thubron EB, Devall M, Soininen H, Kłoszewska I, Mecocci P, Tsolaki M, Vellas B, Schalkwyk L, Dobson R, Malik AN, Powell J, Lovestone S, Hodges A; AddNeuroMed Consortium (2017) Mitochondrial genes are altered in blood early in Alzheimer's disease. *Neurobiol Aging* **53**, 36-47.
- [30] Kobayashi N, Shinagawa S, Nagata T, Shimada K, Shibata N, Ohnuma T, Kasanuki K, Arai H, Yamada H, Nakayama K, Kondo K. Usefulness of DNA Methylation Levels in COASY and SPINT1 Gene Promoter Regions as Biomarkers in Diagnosis of Alzheimer's Disease and Amnesic Mild Cognitive Impairment (2016) *PLoS One* **11**:e0168816.
- [31] Yu L, Chibnik LB, Yang J, McCabe C, Xu J, Schneider JA, De Jager PL, Bennett DA (2016) Methylation profiles in peripheral blood CD4+ lymphocytes versus brain: The relation to Alzheimer's disease pathology. *Alzheimers Dement* **12**, 942-951.
- [32] Gupta VB, Hone E, Pedrini S, Doecke J, O'Bryant S, James I, Bush AI, Rowe CC, Villemagne VL, Ames D, Masters CL, Martins RN; AIBL Research Group (2017) Altered levels of blood proteins in Alzheimer's disease longitudinal study: Results from Australian Imaging Biomarkers Lifestyle Study of Ageing cohort. *Alzheimers Dement (Amst)* **8**, 60-72.
- [33] Knapp MJ, Knopman DS, Solomon PR, Pendlebury WW, Davis CS, Gracon SI (1994) A 30-week randomized controlled trial of high-dose tacrine in patients with Alzheimer's disease. The Tacrine Study Group. *JAMA* **271**, 985-991.
- [34] Jacobs DM, Ard MC, Salmon DP, Galasko DR, Bondi MW, Edland SD (2017) Potential implications of practice effects in Alzheimer's disease prevention trials. *Alzheimers Dement (NY)* **3**, 531-535.

[35] Lewczuk P, Lelental N, Spitzer P, Maler JM, Kornhuber J (2015) Amyloid- $\beta$  42/40 cerebrospinal fluid concentration ratio in the diagnostics of Alzheimer's disease: validation of two novel assays. *J Alzheimers Dis* **43**, 183-91.

[36] Brys M, Pirraglia E, Rich K, Rolstad S, Mosconi L, Switalski R, Glodzik-Sobanska L, De Santi S, Zinkowski R, Mehta P, Pratico D, Saint Louis LA, Wallin A, Blennow K, de Leon MJ (2009) Prediction and longitudinal study of CSF biomarkers in mild cognitive impairment. *Neurobiol Aging* **30**, 682-690.

[37] Nabers A, Perna L, Lange J, Mons U, Schartner J, Guldnhaupt J, Saum KU, Janelidze S, Hollecsek B, Rujescu D, Hansson O, Gerwert K, Brenner H (2018) Amyloid blood biomarker detects Alzheimer's disease. *EMBO Mol Med* Apr 6. pii: e8763. doi: 10.15252/emmm.201708763

[38] Giedraitis V, Sundelöf J, Irizarry MC, Gårevik N, Hyman BT, Wahlund LO, Ingelsson M, Lannfelt L (2007) *Neurosci Lett* **427**,127-131.

[39] Figurski MJ, Waligórska T, Toledo J, Vanderstichele H, Korecka M, Lee VM, Trojanowski JQ, Shaw LM; Alzheimer's Disease Neuroimaging Initiative (2012) The normal equilibrium between CSF and plasma amyloid beta levels is disrupted in Alzheimer's disease. Improved protocol for measurement of plasma  $\beta$ -amyloid in longitudinal evaluation of Alzheimer's Disease Neuroimaging Initiative study patients. *Alzheimers Dement* **8**,250-260.

[40] Teunissen CE, Chiu MJ, Yang CC, Yang SY, Scheltens P, Zetterberg H, Blennow K (2018) Plasma Amyloid- $\beta$  (A $\beta$ 42) Correlates with Cerebrospinal Fluid A $\beta$ 42 in Alzheimer's Disease. *J Alzheimers Dis* **62**,1857-1863.

[41] Thambisetty M, Simmons A, Velayudhan L, Hye A, Campbell J, Zhang Y, Wahlund LO, Westman E, Kinsey A, Güntert A, Proitsi P, Powell J, Causevic M, Killick R, Lunnon K, Lynham S, Broadstock M, Choudhry F, Howlett DR, Williams RJ, Sharp SI, Mitchelmore C, Tunnard C, Leung R, Foy C, O'Brien D, Breen G, Furney SJ, Ward M, Kloszewska I, Mecocci P, Soininen H, Tsolaki M, Vellas B, Hodges A, Murphy DG, Parkins S, Richardson JC, Resnick SM, Ferrucci L, Wong DF, Zhou Y, Muehlboeck S, Evans A, Francis PT, Spenger C, Lovestone S (2010) Association of plasma clusterin concentration with severity, pathology, and progression in Alzheimer disease. *Arch Gen Psychiatry* **67**, 739-748.

[42] Wang P, Chen K, Gu Y, Guo Q, Hong Z, Zhao Q (2017)  $\beta$ -Amyloid Upregulates Intracellular Clusterin but not Secretory Clusterin in Primary Cultured Neurons and APP Mice. *Curr Alzheimer Res* **14**, 1207-1214.

[43] Desikan RS, Thompson WK, Holland D, Hess CP, Brewer JB, Zetterberg H, Blennow K, Andreassen OA, McEvoy LK, Hyman BT, Dale AM; Alzheimer's Disease Neuroimaging

Initiative Group (2014) The role of clusterin in amyloid- $\beta$ -associated neurodegeneration. *JAMA Neurol* **71**, 180-187.

[44] Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, Letenneur L, Bettens K, Berr C, Pasquier F, Fiévet N, Barberger-Gateau P, Engelborghs S, De Deyn P, Mateo I, Franck A, Helisalmi S, Porcellini E, Hanon O; European Alzheimer's Disease Initiative Investigators, de Pancorbo MM, Lendon C, Dufouil C, Jaillard C, Leveillard T, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossù P, Piccardi P, Annoni G, Seripa D, Galimberti D, Hannequin D, Licastro F, Soininen H, Ritchie K, Blanché H, Dartigues JF, Tzourio C, Gut I, Van Broeckhoven C, Alperovitch A, Lathrop M, Amouyel P (2009) Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet* **41**, 1094-1099.

[45] Carrasquillo MM, Belbin O, Hunter TA, Ma L, Bisceglia GD, Zou F, Crook JE, Pankratz VS, Dickson DW, Graff-Radford NR, Petersen RC, Morgan K, Younkin SG (2010) Replication of CLU, CR1, and PICALM associations with Alzheimer disease. *Arch Neurol* **67**, 961-964.

[46] Mielke MM, Hagen CE, Xu J, Chai X, Vemuri P, Lowe VJ, Airey DC, Knopman DS, Roberts RO, Machulda MM, Jack CR Jr, Petersen RC, Dage JL (2018) Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. *Alzheimers Dement* 2018 Apr 5. pii: S1552-5260(18)30067-0.

## 7. TABLES

**Table 1:** Clinical and socio-demographic features of amnesic mild cognitive impairment (aMCI) patients recruited for the IMI-PharmaCog/E-ADNI study. Patients were stratified as CSF A $\beta$ 42/P-tau “positive” and “negative” according to APOE4-specific cut-offs [22].

	“negative” MCI (N = 63)	“positive” MCI (N = 81)	P value <sup>a</sup>
<b>Age, mean (Standard Deviation, SD)</b>	68.3 (8.4)	69.8 (6.3)	0.208
<b>Sex, F/M, No.</b>	36/27	46/35	1.000
<b>Education, mean (SD)</b>	10.0 (4.3)	11.1 (4.4)	0.115
<b>APOE<math>\epsilon</math>4 carriers, No. (%)</b>	3 (5)	63 (78)	<0.001
<b>MMSE, mean (SD)</b>	27.1 (1.8)	26.2 (1.8)	<b>0.006</b>
<b>ADAS-Cog13, mean (SD)<sup>b,c</sup></b>	19.1 (5.9)	21.6 (8.1)	0.052
<b>CSF biomarkers, mean (SD, pg/mL)</b>			
<b>A<math>\beta</math>42</b>	949 (244)	495 (132)	<0.001
<b>P-tau</b>	47 (15)	84 (38)	<0.001
<b>T-tau</b>	301 (149)	614 (394)	<0.001

<sup>a</sup> Parametric t-test (or corresponding non-parametric Mann-Whitney) for continuous Gaussian (or non-Gaussian) distributed variables and Chi-square test for categorical data.

<sup>b</sup> Range 0-85, with 0 as the best score.

<sup>c</sup> Information was missing for 1 patient.

Abbreviations: ADAS-Cog13, Alzheimer Disease Assessment Scale-Cognitive Subscale, version 13; A $\beta$ 42,  $\beta$ -amyloid42; APOE, apolipoprotein E; CSF, cerebrospinal fluid; P-tau, tau phosphorylated at threonine 181; T-tau, total tau.

**Table 2.** IMI-PharmaCog/E-ADNI study patients who underwent plasma assessment.

	“negative” MCI (N = 52)	“positive” MCI (N = 76)	P value <sup>a</sup>
<b>Age, mean (Standard Deviation, SD)</b>	68.2 (8.4)	69.5 (5.9)	0.30
<b>Sex, F/M, No.</b>	26/26	43/33	0.46
<b>Education, mean (SD)</b>	10.0 (4.2)	11.2 (4.5)	0.13
<b>APOE<math>\epsilon</math>4 carriers, No. (%)</b>	2 (3.8)	61 (80)	<b>&lt;0.001</b>
<b>MMSE, mean (SD)</b>	27.0 (1.7)	26.2 (1.8)	<b>0.012</b>
<b>ADAS-Cog13, mean (SD)<sup>b</sup></b>	18.8 (5.7)	21.6 (8.1)	<b>0.033</b>
<b>CSF biomarkers, mean (SD, pg/mL)</b>			
<b>A<math>\beta</math>42</b>	930 (239)	499 (133)	<b>&lt;0.001</b>
<b>P-tau</b>	46 (15)	84 (37)	<b>&lt;0.001</b>
<b>T-tau</b>	295 (146)	619 (397)	<b>&lt;0.001</b>

<sup>a</sup> Parametric t-test (or corresponding non-parametric Mann-Whitney) for continuous Gaussian (or non-Gaussian) distributed variables and by Chi-square test for categorical data.

<sup>b</sup> Range 0-85, with 0 as the best score.

Abbreviations: ADAS-Cog13, Alzheimer Disease Assessment Scale-Cognitive Subscale, version 13; A $\beta$ 42,  $\beta$ -amyloid42; APOE, apolipoprotein E; CSF, cerebrospinal fluid; P-tau, tau phosphorylated at threonine 181; T-tau, total tau.

**Table 3.** Linear Mixed Models for the analysis of selected plasma molecules in aMCI patients stratified as “positive”, as prodromal AD, and “negative” as a control group, according to cut-offs of CSF A $\beta$ 42/P-tau [22]. The model included age, sex, baseline MMSE score, Time, Group (A $\beta$ 42/P-tau status), and Time X Group interaction as predictors. Significant (P-value < 0.05) effects are shown in bold.

Measure (dependent variable)	Time		Group		Time X Group		Pseudo $\eta^2$ (Effect size)
	Std $\beta$	P-value	Std $\beta$	P-value	Std $\beta$	P-value	
A $\beta$ 42	0.209	<b>&lt;.0001</b>	0.011	0.937	0.151	<b>0.048</b>	0.25
A $\beta$ 40	0.206	<b>0.009</b>	0.142	0.286	0.036	0.815	0.01
A $\beta$ 42/ A $\beta$ 40	0.193	<b>.0006</b>	0.062	0.725	0.147	0.326	0.01
CLU	0.462	<b>&lt;0.001</b>	0.085	0.562	0.062	0.663	0.01

Abbreviations: Std  $\beta$ , standardized  $\beta$  coefficient of Linear Mixed Model; CLU: clusterin (apolipoprotein J).

**Table 4.** Longitudinal Mixed Model Analysis of the association between cognitive decline (ADAS-Cog 13, dependent variable) and peripheral circulating molecules in the whole group and in the A $\beta$ 42/P-tau positive MCI patients [22]. Significant (p< 0.05) effects are shown in bold.

Measure (independent variable)	Whole MCI group		A $\beta$ 42/P-tau positive MCI patients	
	Biomarker		Biomarker	
	Standardized $\beta$	P-value	Standardized $\beta$	P-value
A $\beta$ 42	0.267	<b>0.003</b>	0.225	<b>0.046</b>
A $\beta$ 40	0.047	0.346	0.079	0.150
A $\beta$ 42/ A $\beta$ 40	0.225	<b>0.002</b>	0.226	<b>0.016</b>
CLU	0.149	<b>0.002</b>	0.096	0.092

Abbreviations. CLU: clusterin (apolipoprotein J).

## 8. FIGURE LEGENDS

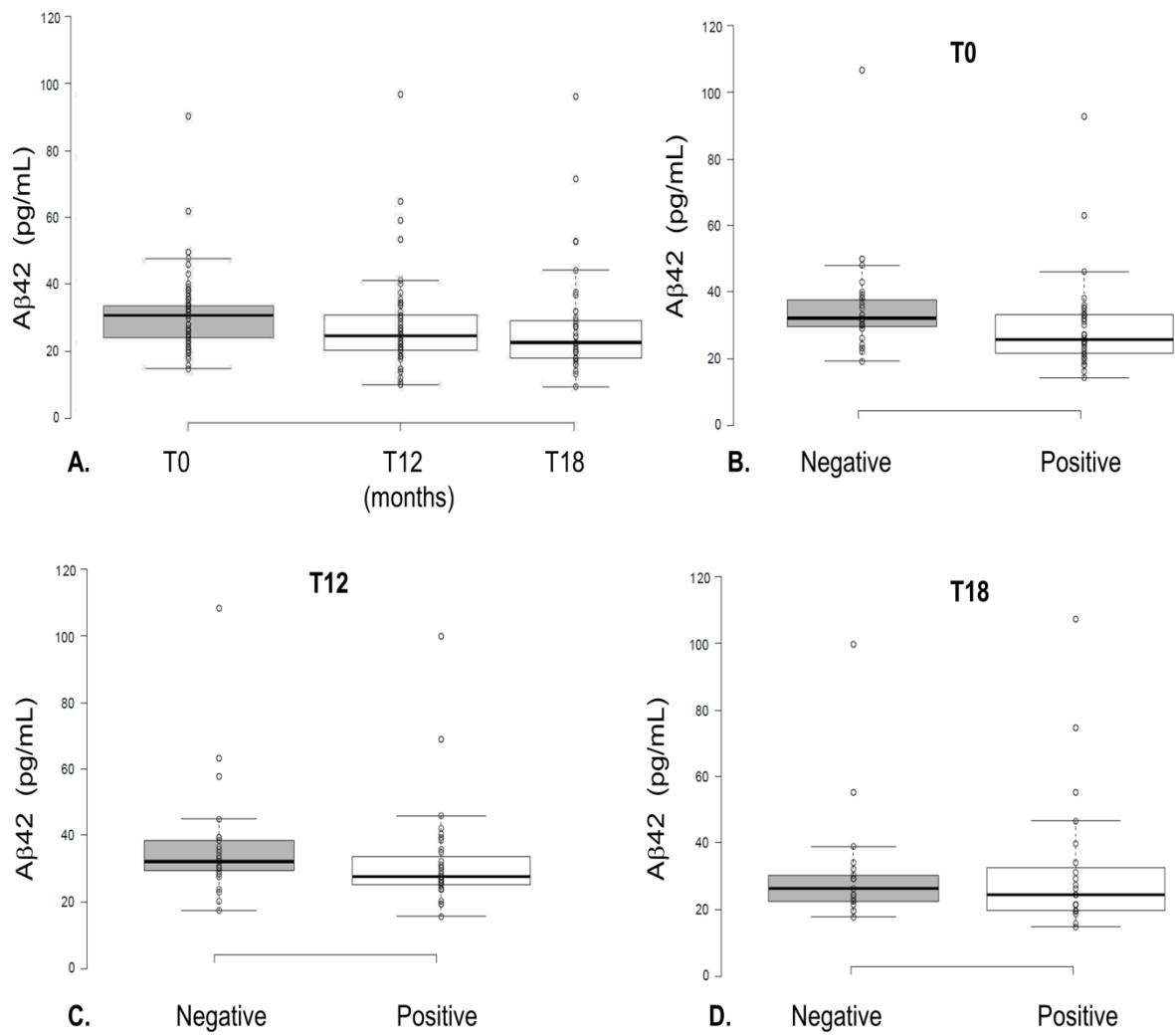
**Figure 1. Plasma A $\beta$ 42 levels in the IMI-PharmaCog/E-ADNI study.** (A) Whole aMCI group over time; (B) Baseline (T0); (C) Assessment after 12 months from T0 (T12); (D) Assessment 18 months from T0 (T18). Data are presented as box-plot, with the upper box line indicating the 3rd quartile, the lower one the 1st quartile and the bold line the median. The single measures are also indicated as empty circles. “Negative” and “positive” refer to the classification of aMCI according to the APOE-specific cut-offs [22].

**Figure 2. Plasma A $\beta$ 40 levels in the IMI-PharmaCog/E-ADNI study.** (A) Whole aMCI group over time; (B) Baseline (T0); (C) Assessment 12 months from T0 (T12); (D) Assessment 18 months from T0 (T18). Data are presented as box-plots, with the upper line indicating the 3rd quartile, the lower one the 1st quartile and the bold line the median. The single measures are also indicated as empty circles. “Negative” and “positive” refer to the classification of aMCI according to the calculated algorithm, as reported above.

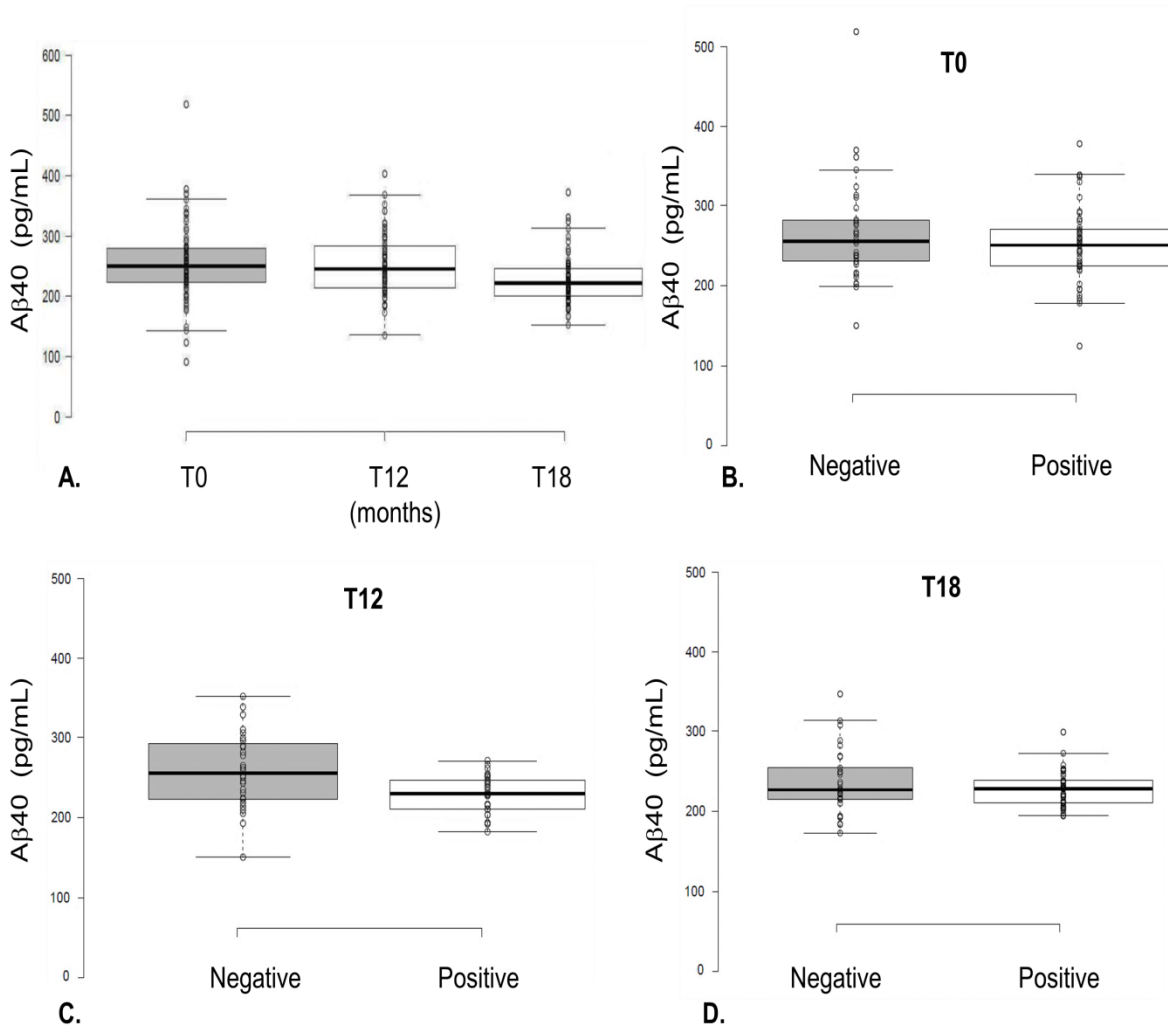
**Figure 3. Plasma A $\beta$ 42/A $\beta$ 40 ratios in the IMI-PharmaCog/E-ADNI study.** (A) Whole aMCI group over time; (B) Baseline (T0); (C) Assessment 12 months from T0 (T12); (D) Assessment 18 months from T0 (T18). Data are presented as box-plots, with the upper line indicating the 3rd quartile, the lower one the 1st quartile and the bold line the median. The single measures are also indicated as empty circles. “Negative” and “positive” refer to the classification of aMCI as already described.



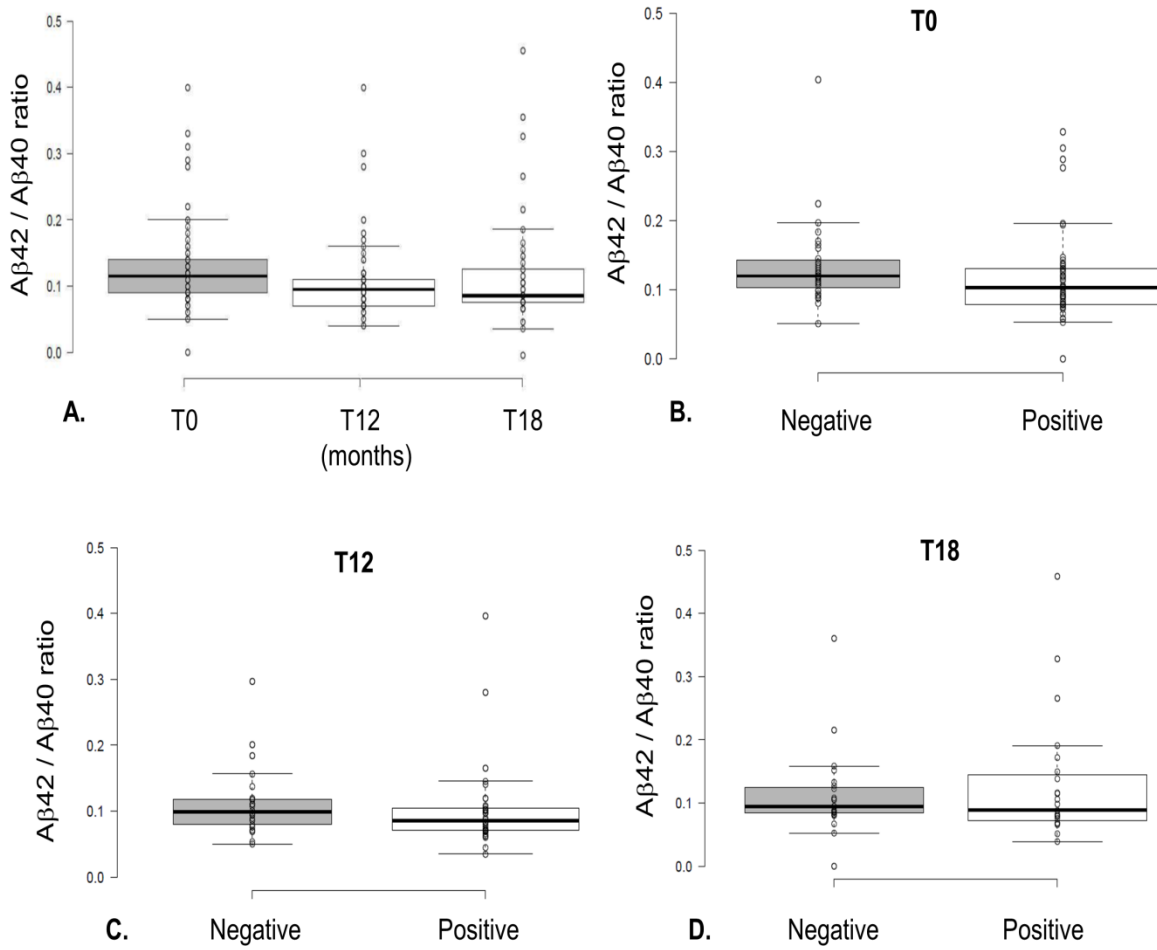
**Figure 4. Plasma clusterin (CLU) in the IMI-PharmaCog/E-ADNI study.** (A) Whole aMCI group over time; (B) Baseline (T0); (C) Assessment 12 months from T0 (T12); (D) Assessment 18 months from T0 (T18). Data are presented as box-plots, with the upper line indicating the 3rd quartile, the lower one the 1st quartile and the bold line the median. The single measures are indicated as empty circles. For “negative” and “positive” aMCI classification see above. \*\*\* $p < 0.001$  vs. T0, ANOVA and Tukey’s post-hoc test.



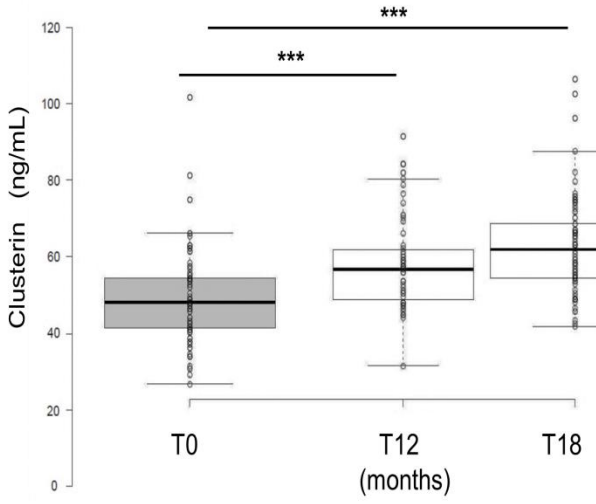
**Figure 1**



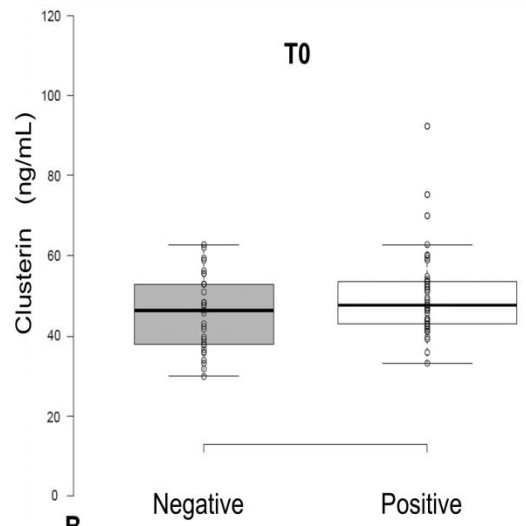
**Figure 2**



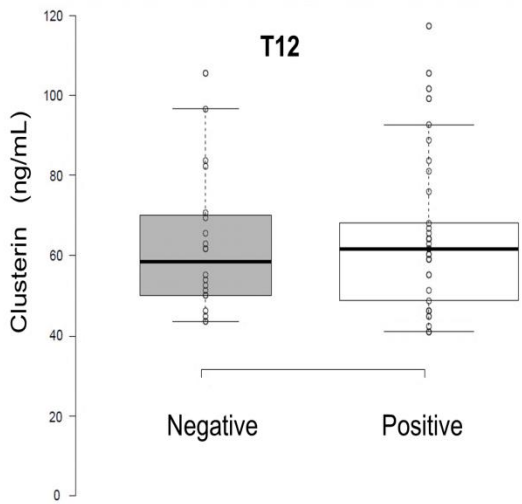
**Figure 3**



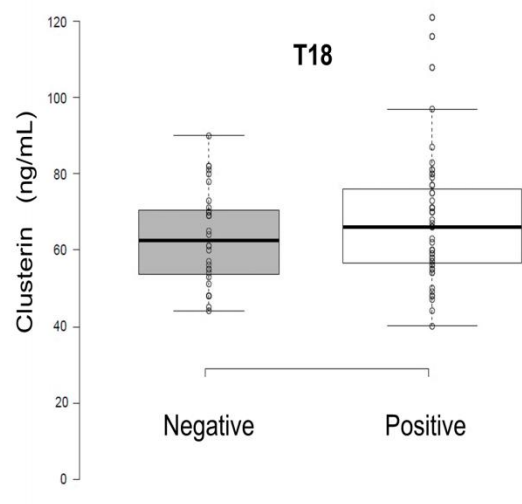
**A.**



**B.**



**Figure 4 C.**



**D.**