



HAL
open science

Plasma Amyloid Beta Predicts Conversion to Dementia in Subjects with Mild Cognitive Impairment: The BALTAZAR Study

Olivier Hanon, Jean-Sébastien Vidal, Sylvain Lehmann, Stéphanie Bombois,
Bernadette Allinquant, Christiane Baret-Rose, Jean-Marc Tréluyer, Hendy
Abdoul, Patrick Gelé, Christine Delmaire, et al.

► To cite this version:

Olivier Hanon, Jean-Sébastien Vidal, Sylvain Lehmann, Stéphanie Bombois, Bernadette Allinquant, et al.. Plasma Amyloid Beta Predicts Conversion to Dementia in Subjects with Mild Cognitive Impairment: The BALTAZAR Study. *Alzheimer's & Dementia: the Journal of the Alzheimer's Association*, 2022, 18, pp.2537-2550. 10.1002/alz.12613 . hal-03719721

HAL Id: hal-03719721

<https://u-picardie.hal.science/hal-03719721v1>

Submitted on 7 Jul 2023

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 amyloid β predicts conversion to dementia in subjects with mild cognitive impairment. The BALTAZAR study

Olivier Hanon [1], Jean-Sébastien Vidal [1], Sylvain Lehmann [2], Stéphanie Bombois [3], Bernadette Allinquant [4], Christiane Baret-Rose [4], Jean-Marc Tréluyer [5], Hendy Abdoul [5], Patrick Gelé [6], Christine Delmaire [3], Frédéric Blanc [7], Jean-François Mangin [8], Luc Buée [3], Jacques Touchon [9], Jaques Hugon [10], Bruno Vellas [11], Evelyne Galbrun [12], Athanase Benetos [13], Gilles Berrut [14], Elena Paillaud [15], David Wallon [16], Giovanni Castelnovo [17], Lisette Volpe-Gillot [18], Marc Paccalin [19], Philippe Robert [20], Olivier Godefroy [21], Vincent Camus [22], Joël Belmin [23], Pierre Vandell [24], Jean-Luc Novella [25], Emmanuelle Duron [26], Anne-Sophie Rigaud [1], Susanna Schraen-Maschke [3]*, Audrey Gabelle [9]* on behalf of the BALTAZAR study group.

Affiliations

[1] Université de Paris, EA 4468, APHP, Hopital Broca, Memory Resource and Research Centre of de Paris-Broca-Ile de France, F-75013 Paris, France.

[2] Université de Montpellier, CHU Montpellier, LBPC, Inserm, F-34000 Montpellier, France.

[3] Univ. Lille, Inserm, CHU Lille, U1172-LiNCog, LICEND, LabEx DISTALZ, F-59000 Lille, France.

[4] Université de Paris, Institute of Psychiatric and Neurosciences, Inserm UMR-S 1266, F-75014 Paris, France.

[5] Université de Paris, APHP, Hôpital Necker, Clinical Research Unit, F-75015 Paris, France.

[6] Univ. Lille, Inserm, CHU Lille, CRB/CIC1403, F-59000 Lille, France.

[7] Université de Strasbourg, Hôpitaux Universitaires de Strasbourg, CM2R, pôle de Gériatrie, Laboratoire ICube, FMFS, CNRS, équipe IMIS, F-67000 Strasbourg, France.

[8] Université Paris-Saclay, Neurospin, CEA, CNRS, cati-neuroimaging.com, CATI Multicenter Neuroimaging Platform, F-91190 Gif-sur-Yvette, France.

[9] Université de Montpellier, CHU Montpellier, Memory Research and Resources center of Montpellier, department of Neurology, Inserm INM NeuroPEPs team, excellence center of neurodegenerative disorders, F-34000 Montpellier, France.

[10] Université de Paris, APHP, Groupe Hospitalier Saint Louis-Lariboisière-Fernand Widal, Center of Cognitive Neurology, F-75010 Paris, France.

[11] Université de Toulouse III, CHU La Grave-Casselardit, Memory Resource and Research Centre of Midi-Pyrénées, F-31300 Toulouse, France.

[12] Sorbonne Université, APHP, Centre Hospitalier Dupuytren, Department of Gérontology 2, F-91210 Draveil, France.

[13] Université de Lorraine, CHRU de Nancy, Memory Resource and Research Centre of Lorraine, F-54500 Vandoeuvre-lès-Nancy, France.

[14] Université de Nantes, EA 4334 Movement-Interactions-Performance, CHU Nantes, Memory Research Resource Center of Nantes, Department of clinical gerontology, F-44000 Nantes, France.

[15] Université de Paris, APHP, Hôpital européen Georges Pompidou, Service de Gériatrie, F-75015, Paris, France.

- [16] Normandie Univ, UNIROUEN, Inserm U1245, CHU de Rouen, Department of Neurology and CNR-MAJ, Normandy Center for Genomic and Personalized Medicine, CIC-CRB1404, F-76000, Rouen, France.
- [17] CHU de Nimes, Hôpital Caremeau, Neurology Department, F-30029 Nimes, France.
- [18] Hôpital Léopold Bellan, Service de Neuro-Psycho-Gériatrie, Memory Clinic, F-75014 Paris, France.
- [19] CHU de Poitiers, Memory Resource and Research Centre of Poitiers, F- 86000 Poitiers, France.
- [20] Université Côte d’Azur, CHU de Nice, Memory Research Resource Center of Nice, CoBTek lab, F-06100 Nice, France.
- [21] CHU d’Amiens-Picardie, Memory Resource and Research Centre of AmiensPicardie, F-80000 Amiens, France.
- [22] Université François-Rabelais de Tours, CHRU de Tours, UMR Inserm U1253, F-37000 Tours, France.
- [23] Sorbonne Université, APHP, Hôpitaux Universitaires Pitie-Salpêtrière-Charles Foix, Service de Gériatrie Ambulatoire, F-75013 Paris, France.
- [24] Université Bourgogne Franche-Comté, Laboratoire de Recherches Inté- gratives en Neurosciences et Psychologie Cognitive, CHU de Besançon, Memory Resource and Research Centre of Besançon Franche-Comté, F-25000 Besançon, France.
- [25] Université de Reims Champagne-Ardenne, EA 3797, CHU de Reims, Memory Resource and Research Centre of Champagne-Ardenne, F-51100 Reims, France.
- [26] Université Paris-Saclay, APHP, Hôpital Paul Brousse, département de gériatrie, Équipe MOODS, Inserm 1178, F-94800 Villejuif, France.

Corresponding author:

Prof. Olivier Hanon, Hôpital Broca, Service de Gériatrie, 54-56 rue Pascal – 75013 Paris, France

Key words: plasma biomarkers; amyloid beta protein, prognosis; mild cognitive impairment; major neurocognitive disorder; minor neurocognitive disorder; Alzheimer’s disease; CSF.

* Codirected this work

Abstract

INTRODUCTION, Blood-based biomarkers are the next challenge for Alzheimer's disease (AD) diagnosis and prognosis.

METHODS, MCI participants (N=485) of BALTAZAR study, a large-scale longitudinal multicenter cohort, were followed-up for 3 years. 165 of them converted to dementia (95% AD). Associations of conversion and plasma $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-42}/A\beta_{1-40}$ ratio were analyzed with logistic and Cox models.

RESULTS, Converters to dementia had lower level of plasma $A\beta_{1-42}$ (37.1 (12.5) vs. 39.2 (11.1), P value=0.03) and lower $A\beta_{1-42}/A\beta_{1-40}$ ratio than non-converters (0.148 (0.125) vs. 0.154 (0.076), P value=0.02). MCI participants in the highest quartile of $A\beta_{1-42}/A\beta_{1-40}$ ratio (≥ 0.169) had a significant lower risk of conversion (Hazard Ratio adjusted for age, sex, education, $APOE \epsilon 4$, hippocampus atrophy = 0.52 (95%CI [0.31-0.86], P value=0.01).

DISCUSSION, In this large cohort of MCI subjects we identified a threshold for plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio that may detect patients with a low risk of conversion to dementia within 3 years.

Background

Blood-based biomarkers are the next challenge for Alzheimer's disease (AD) diagnosis, prognosis and prediction. Since 2007, cerebrospinal fluid (CSF) amyloid β ($A\beta$)₁₋₄₂ or $A\beta$ ₁₋₄₂/ $A\beta$ ₁₋₄₀ ratio, Tau, p-Tau, PET-amyloid biomarkers are used as pathophysiological biomarkers, in combination with topographical biomarkers (clinical, neuropsychological and brain imaging) to establish the diagnosis of AD in research [1] and in daily clinical practice [2-6]. Their capacity to detect early AD and individuals at risk to develop AD from mild cognitive impairment (MCI), cognitive complaint or asymptomatic familial history of AD cases [7-10] has already been demonstrated. With the development of targeted drugs and preventive strategies prior to cognitive decline, non-invasive, reproducible and easy-to-use tools need to be validated in order to prescreen subjects at risk of AD, of conversion to dementia.

More recently, plasma $A\beta$ ₁₋₄₂ or $A\beta$ ₁₋₄₂/ $A\beta$ ₁₋₄₀ ratio have been found significantly correlated to CSF $A\beta$ ₁₋₄₂ or CSF $A\beta$ ₁₋₄₂/ $A\beta$ ₁₋₄₀ ratio [11-13]. Plasma $A\beta$ ₁₋₄₂ and $A\beta$ ₁₋₄₂/ $A\beta$ ₁₋₄₀ ratio also predict PET amyloid positivity with good sensitivity and specificity among elderly cognitive normal subjects, MCI and AD patients (AUC from 0.72 to 0.98) [13-21]. However, the prognosis relevance of plasma $A\beta$ biomarkers remained to be fully validated. Studies of plasma $A\beta$ levels assessing the interest of plasma $A\beta$ biomarkers yielded contradictory results [22]. Lastly low plasma $A\beta$ ₁₋₄₂ or $A\beta$ ₁₋₄₂/ $A\beta$ ₁₋₄₀ ratio has found associated with conversion to dementia in some large community-based cohorts [17, 23-26]. In other cohorts however, this association was not confirmed [27-29].

In addition to $A\beta$ ₁₋₄₂ and $A\beta$ ₄₀, soluble amyloid precursor protein α (sAPP α) and β (sAPP β) are promising novel biomarkers in CSF of AD patients [30, 31] and more recently in blood [32]. However, these results were not confirmed in other studies [33, 34]. A transient increase in sAPP α secretion in response to $A\beta$ ₁₋₄₂ oligomers has been observed suggesting that sAPP α levels may vary with the clinical staging of patients [35].

We investigated whether plasma concentrations of $A\beta$ ₁₋₄₂, $A\beta$ ₁₋₄₀, $A\beta$ ₁₋₄₂/ $A\beta$ ₁₋₄₀ ratio and sAPP α , predict the conversion from MCI to dementia, in particular AD, over 3 years of follow-up in the large-scale longitudinal multicenter BALTAZAR (Biomarker of AmyLoid pepTide and ALZheimer's disease Risk) cohort.

Methods

Participants

The MCI participants (n=539) were selected from the BALTAZAR study, a multicenter (23 French memory centers) prospective cohort (ClinicalTrials.gov Identifier #NCT01315639) with a 3-year follow-up. BALTAZAR protocol has already been published [12]. MCI diagnosis was based on Petersen's criteria [36, 37]. Participants with Lewy Body, Parkinson, frontotemporal or vascular MCI disorders were excluded in order to maximize the likelihood of conversion to Alzheimer's disease.

All participants were Caucasian, community-dwellers and had caregivers. At baseline, all the participants underwent clinical, neuropsychological and biological assessments and in participants unless contradictions, brain MRI. CSF samples were collected in accepting participants.

Cognitive evaluations were performed with extensive neuropsychological battery by neuropsychologists after training program to harmonize the quotation [12]. MCI subjects at baseline were dichotomized into amnesic (a-MCI) and non-amnesic (na-MCI) phenotypes according to the presence of memory impairment on FCSRT related to age, sex and educational level. Six neuropsychological follow-up visits were scheduled at 6 months (M6), 12 months (M12), 18 months (M18), 24 months (M24), 30 months (M30) and 36 months (M36), with ADL and IADL and CDR and comprehensive neuropsychological battery tests including the MMSE. At the end of each follow-up visit, MCI participants were classified into MCI stage or into conversion to dementia stage.

Because diagnostic criteria have evolved and now includes biomarkers [38], we also classified MCI participants in MCI core-clinical, MCI low-likelihood, MCI intermediate-likelihood and MCI high-likelihood groups, based on the positivity of A β biomarkers such as CSF A β 42 and neuronal injury based on CSF Tau or MRI hippocampal atrophy. MCI were then classified according to the ATN classification [39] and the PLM scale [4] and Albert' criteria [40].

All participants gave written informed consent to participate in the study. The BALTAZAR study was approved by the Paris ethics committee (CPP Ile de France IV Saint-Louis Hospital).

Determination of Conversion from MCI to dementia

The conversions from MCI to AD dementia were reviewed by an adjudication committee only based on the clinical and neuropsychological characteristics by using the NIA-AA criteria blinded to CSF and plasma biomarker results [38]. Decline in cognitive functioning and disability in activities of daily living, measured by changes from baseline in scores of the MMSE, IADL, ADL, and CDR (≥ 1), defined the progression from MCI to dementia.

Biomarkers

Protocol used for the measurements of CSF and plasma biomarkers has already been published [12]. Briefly analyses of plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ and CSF $A\beta_{1-42}$, Tau and p-Tau were performed in a single centralized laboratory with the same collection tubes across all study centers (low binding Eppendorf® (LoBind microtubes Eppendorf, ref 022431064, Hamburg, Germany)).

Plasma $A\beta$ peptide assay was performed using the INNO-BIA kit (Fujirebio Europe NV, formerly Innogenetics NV, Belgium), based on a multiplex xMAP technique with a LABScan-200 system (Luminex BV, The Netherlands). CSF $A\beta_{42}$, total-tau and phosphorylated-tau (p-tau₁₈₁) levels were measured using commercially available Innotest® sandwich ELISA INNOTEST® hTAU Ag, INNOTEST® β -Amyloid₍₁₋₄₂₎ and INNOTEST® Phospho-tau_(181P) according to the manufacturer's procedures (Fujirebio Europe NV, formally Innogenetics NV, Belgium).

For sAPP α , serum was collected on Venosafe® plastic tubes VF-076SP and the same Eppendorf tubes were used for aliquots. sAPP α was evaluated using our previous test based on homogenous time-resolved fluorescence [41].

MRI brain imaging

The MRI protocol has already been published [12]. included a 3D volumetric T1 weighted, an axial FLAIR T2W, an axial EG T2W, an axial T2W FSE, an axial blood oxygen-level dependence (BOLD) echo planar EPI (10 min); an axial diffusion tensor imaging (DTI); and an arterial spin labeling imaging. After MRI were completed the scans were sent for quality validation and post-processing. MRI analysis was centralized and analyzed by the CATI (Centre d'acquisition et de traitement d'images) [42]. Hippocampal atrophy was assessed using Scheltens scale on baseline brain MRI.

Other confounding factors

The standardized interview included questions on demographic and socioeconomic characteristics, health status and medication use of the participants. Diabetes was defined as a self-reported diagnosis of diabetes or fasting blood glucose > 7.0 mmol/L or use of glucose-modifying medication and hypertension as a measured systolic blood pressure \geq 140 mmHg or diastolic blood pressure \geq 90 mmHg or current antihypertensive treatment. Disability was assessed using IADL (Instrumental Activities of Daily Living, normal score 14/14) and ADL (Activities of Daily Living, normal score 6/6) scales. Depressive symptoms were evaluated with the GDS (Geriatric Depression Scale).

APOE was genotyped in a single centralized laboratory [12].

Statistical analyses

In BALTAZAR cohort among the 539 MCI participants, 485 had available plasma biomarkers. Because plasma $A\beta_{1-40}$, $A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratio did not have a normal distribution, log transformed variables were used in statistics for comparison but for the sake of clarity, non-transformed values are presented in the tables. General characteristics were analyzed in the whole sample and according to conversion to dementia. Categorical variables are presented as percentage and counts (% (N)), continuous variables as mean and standard deviation (M (SD)) and comparison were made with χ^2 tests and T-tests respectively. Because $A\beta_{1-42}/A\beta_{1-40}$ ratio did not have a normal distribution, quartiles of $A\beta_{1-42}/A\beta_{1-40}$ ratio were computed and Kaplan Meier curves were drawn for conversion to dementia according to quartiles of $A\beta_{1-42}/A\beta_{1-40}$ ratio and overall difference between the quartiles was calculated with Log rank test. General characteristics were also analyzed according to highest quartile of $A\beta_{1-42}/A\beta_{1-40}$ ratio vs. three lower quartiles. Relationship between conversion and plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio was assessed with Cox regression models with conversion as dependent variables and being in the highest quartile of plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio as independent variables adjusted for age, sex, education level, presence of *APOE* $\epsilon 4$ -allele, MMSE score at baseline and hippocampus atrophy. The relationship was also analyzed with general logistic regression model. We also examined how plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio improved dementia risk prediction of a models with age, sex, education level and *APOE* $\epsilon 4$ by calculating continuous net reclassification improvement (NRI) and integrated discrimination improvement (IDI) using bootstrap (IDI \approx 0.004 or NRI \approx 0.16: small improvement IDI \approx 0.024 and NRI \approx 0.40: moderate improvement IDI \approx 0.06 and NRI \approx 0.62: large improvement) [43].

A receiver operating characteristic (ROC) curves was performed to compare sensitivity and specificity of plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio for predicting conversion to dementia. Area under curve (AUC) and confidence interval of 95% were estimated using bootstrap technique (1000 iterations).

All analyses were done using R software (R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). In all analyses, the 2 sided α -level of 0.05 was used for significance testing.

Results

Characteristics of the participants at baseline and within the follow-up

At baseline, among the 485 participants with clinical MCI and plasma biomarkers available, mean age was 77.7 (5.5), 60.4% (n=293) were women and 48% (n=232) had at least high school diploma. On average MMSE score was 26.4 (2.5) and 39.6% (191) were *APOE* ϵ 4 carriers. At baseline 77% (n=373) had amnesic form of MCI and 19% (n=91) had MCI due to AD with high likelihood according to the Albert's diagnosis criteria because they had low $A\beta$ level and neurodegenerative lesions of AD, and 27% MCI with A+T+N+ and PLM scale 3.

During the clinical follow-up period of 6-36 months, 30% (n=145) of the MCI participants developed dementia on average 15.0 (8.4) months after baseline visit and in 95% of the cases, they converted to probable AD.

During the 3-year follow-up, 36.9% (n=179) of participants were lost to follow-up: 8.4 % (n=41) after the baseline visit, 5.8% (n=28) after the 6-month visit, 3.3% (n=16) after the 12-month visit, 3.3% (n=16) after the 18-month visit, 15.1% (n=73) after the 24-month visit, 1.0% (n=5) after the 30-month visit. Moreover, 15 participants died during the 3-year follow-up.

The converters to dementia were at baseline older (78.6 (5.7) vs. 77.4 (5.4) years, P value=0.03), they scored poorer to cognitive testing with a lower MMSE score (25.6 (2.5) vs. 26.8 (2.5), P value<.0001), they had more frequently amnesic MCI (92.4% vs. 70.3%, P value<.0001) and they were more often *APOE* ϵ 4 carriers (56.6% vs. 32.3%, P value<.0001) (table 1). Moreover, the converters had more frequently hippocampal atrophy (P value<.001) (table 1). All these results remained significant after adjustment for age, sex, educational level and the *APOE* ϵ 4 status (table 1). These results remained the same for converters to probable dementia.

Analysis of CSF biomarkers profile showed a typical AD profile in converters to dementia compare to non-converters with a lower $A\beta_{1-42}$ level, higher Tau and p-Tau levels and a higher p-Tau/ $A\beta_{1-42}$ ratio (P value<.0001 for all) (table 2). Only one (1.9%) MCI with low likelihood to be due to AD converted to AD.

No significant differences in CSF sAPP α levels were observed between converters and non-converters (table 2).

Conversion to dementia and plasma biomarkers

Converters to dementia had a lower level of plasma $A\beta_{1-42}$ at baseline than non-converters (37.1 (12.5), vs. 39.2 (11.1) P value=0.03) and a lower $A\beta_{1-42}/A\beta_{1-40}$ ratio than non-converters (0.148 (0.125)

vs. 0.154 (0.076), P value=0.02) (table 2). No significant difference was observed for the plasma $A\beta_{1-40}$ or sAPP α concentrations.

The relationship between conversion and plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio was not linear: 18.2% (22/121) of participants converted to dementia in the highest quartile (plasma $A\beta_{1-42}/A\beta_{1-40} \geq 0.169$), 33.1% (40/121) in the third quartile ($0.144 \geq$ plasma $A\beta_{1-42}/A\beta_{1-40} > 0.169$), 29.8% (36/121) in the 2nd quartile ($0.119 \geq$ plasma $A\beta_{1-42}/A\beta_{1-40} > 0.144$) and 38.5% (47/122) in the lowest quartile (plasma $A\beta_{1-42}/A\beta_{1-40} \leq 0.119$) (table 2). Among the highest quartile, 22 (18.1%) converted to dementia while 123 (33.9%) converted to dementia in the three lower quartiles (P value=.001) (table 3). The Kaplan Meier curves also showed a significant lower risk of conversion in the highest quartile vs. the three lower quartiles (P value=0.002) (figure 1). Demographic, clinical and MRI characteristics of MCI participants in the highest quartile were not significantly different than those in the three lower quartiles except that they were less likely *APOE* $\epsilon 4$ allele carriers (table 3). In addition, in participants in the highest quartile of plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio, the CSF biomarkers profile was quite normal with higher levels of $A\beta_{1-42}$ (P value=0.01) and lower levels of Tau and p-Tau (P value=0.003 and P value=0.0009 respectively) (table 3). Finally participants in the highest quartile had less frequently the high likelihood MCI profile (P value=0.005) (table 3).

Even in participants in the PLM three strata (with pathological values in three CSF biomarkers at baseline), those in the highest quartile of plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio (plasma $A\beta_{1-42}/A\beta_{1-40} > 0.169$) (n=3) did not convert to dementia.

In a multivariate Cox model with conversion status as dependent variable, being in the highest quartile was associated with a lower risk of conversion (HR = 0.52 (0.31-0.86), P value=0.01) with adjustment for age, sex, education level, *APOE* $\epsilon 4$ status, MMSE score at baseline and hippocampus atrophy (table 4). Similar result was obtained using logistic regression (HR = 0.45 (0.23-0.82), P value=0.01) (table 4) with the same adjustment.

With a threshold of plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio set at 0.169, the sensitivity of prediction of non-conversion was 0.848 and specificity was 0.297. The AUC for plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio was 0.587 (95% CI 0.531-0.642).

Improvement of conversion risk with plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio

Addition of plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio (highest quartile vs. three lower quartiles) to a model including age, sex, education level and *APOE* $\epsilon 4$ status significantly improved the prediction of conversion to dementia of MCI participants (table 5). However the improvement of IDI for the highest quartile vs. the three lower quartiles of $A\beta_{1-42}/A\beta_{1-40}$ ratio was relatively small with an IDI of 0.018 (0.007; 0.030) (P value=0.0009), and the improvement of NRI was moderate with a NRI of 0.274 (0.122; 0.426) (P value=0.0004) (table 5).

Discussion

In this large-scale multicenter longitudinal cohort of clinically defined MCI referred to memory center with repeated extensive neuropsychological testing and plasma, CSF and MRI biomarkers, MCI participants that convert to dementia had lower levels of plasma $A\beta_{1-42}$, and $A\beta_{1-42}/A\beta_{1-40}$ ratio compared to non-converters independently of age, sex, education level and *APOE* $\epsilon 4$. More interestingly, the MCI participants among the highest quartile of the $A\beta_{1-42}/A\beta_{1-40}$ ratio (≥ 0.169) had a significant 48% reduction of risk to develop dementia and AD independently of age, sex, education level and *APOE* $\epsilon 4$, MMSE score at baseline and hippocampus atrophy.

Interestingly, participants in the highest quartile of plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio were not significantly different than those in the three lower quartiles in terms of demographic, clinical and neuropsychological characteristics except for *APOE* $\epsilon 4$ carriers, highlighting the potential interest of this plasmatic ratio in differentiating MCI converters from non-converters.

Among the numerous heterogeneous cohort studies (including normal cognitive controls, subjective cognitive complaint), only a few of them evaluated plasma $A\beta$ levels as biomarker of conversion to dementia in MCI population. One large MCI cohort (N= 588) already found that plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio was associated with conversion to AD after a follow-up of 4-6 years [44]. Another study focused only on amnesic MCI (42 stable aMCI and 37 aMCI-AD, mean 75 years old) and found a significant correlation between higher $A\beta_{1-42}$ and $A\beta_{1-40}$ levels and conversion to AD, but not for $A\beta_{1-42}/A\beta_{1-40}$ ratio [45].

Conversely, in two independent Swedish cohorts (mean age 70 and 63y) plasma $A\beta_{1-42}$, $A\beta_{1-40}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratio were not different between MCI participants that converted to AD and those that did not, over a follow-up of 4-7 years and 2-4 years respectively [29]. However, these 2 cohorts were of small size (n=117 and 110) and had relatively low conversion rates. In addition the mean levels of $A\beta_{1-42}/A\beta_{1-40}$ ratio were high among MCI participants (0.15 in cohort A; and 0.20 in cohort B) comparable to our highest quartile threshold (0.169 with the same method used) suggesting that there might have been a recruitment bias in favor of MCI subjects with high $A\beta_{1-42}/A\beta_{1-40}$ ratio and low conversion rate. All together this could explain the lack of association. Another study including a small number of older MCI subjects (9 stable MCI and 33 MCI-dementia, mean age 80y) with a mean follow-up of 4.5 years, did not find any relationship between plasma $A\beta_{1-42}$, $A\beta_{1-40}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratio and conversion [46]. In the GEMS cohort [27] including normal controls and MCI, low plasma $A\beta_{1-42}$ and low $A\beta_{1-42}/A\beta_{1-40}$ ratio, were associated with dementia risk over 8.5 years of follow-up only in normal control participants, but not in MCI participants.

All these inconsistent results may be related to the heterogeneity of MCI population and to the fact that these studies used different techniques for plasma $A\beta$ measurements (Luminex technology,

different commercial ELISA kits or home-made ELISA) although in BALTAZAR study, all measurements were processed with the same protocol in a unique centralized laboratory.

Meanwhile, even if our results are consistent with most of the studies showing a relationship between $A\beta_{1-42}$ and risk of dementia, the difference between levels of MCI converters and non-converters is not large enough to be used alone as a good predictor of dementia or AD risk. However in the current study, MCI participants in the highest quartile of the $A\beta_{1-42}/A\beta_{1-40}$ ratio had a 48%-reduction of risk to develop dementia and AD and only three MCI participants (6%) were A+T+N+ while 38% were A-T-N-. In a large MCI cohort (N=643) [47], plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio above 0.180 was associated with low risk of AD, through an AD surrogate phenotype defined by CSF p-Tau > 70 pg/mL. This threshold can be compared with our highest quartiles of plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio (> 0.169). In our study the three lower quartiles had the same risk of conversion in our study. Moreover, among the highest quartile, the three MCI with A+T+N+ did not convert to AD during the follow-up. In clinical practice, a plasma A β ratio above 0.169 could identify with a good sensitivity MCI with low risk of conversion. However the low specificity and moderate NRI preclude its use as diagnostic tool. It could however be used as a non-invasive first step screening tool in protocols requiring MCI patients with CSF AD phenotype and rapid conversion to AD or to exclude patients with weak probability to decline during the clinical trials.

Addition of plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio to a logistic model including age, sex, education level and presence of *APOE* $\epsilon 4$ allele improved moderately the prediction of conversion of MCI participants probably due to the importance of the effect of *APOE* $\epsilon 4$ allele that is associated with greater amyloid brain load [48]. Although, there was a difference in A β levels in MCI that converted to dementia and AD, the difference is not sufficient to be used as diagnostic tool in clinical practice.

To explain that the three lower quartiles had equivalent risk to convert to dementia and significantly higher than the highest quartile, one could hypothesized that, compared to other population-based cohorts like the Rotterdam study, AIBL or BioFinder cohorts, the MCI subgroup of the BALTAZAR study was more homogeneous with only patients with cognitive impairment. Therefore a large proportion of them were probably already engaged in the AD pathology as suggested by the high level of conversion to dementia (30%) over the 3-year follow-up.

The strength of our study lies in the large number of well-characterized MCI participants with repeated extensive standardized cognitive tests. All the biochemical analyses were centralized in a single laboratory, eliminating inter-center variability. To limit measurement bias, the same pre-analytical protocol was followed throughout the study, with shipment to all centers of the same collection tubes. All measurements were processed blind to the participants' diagnosis, MRI and CSF results. The same lot was used for measurement of plasma A β levels at inclusion. We used the

commercially available Luminex technology that has good analytical performances and is already used in routine [49]. In addition, the brain MRI was analyzed in a single dedicated expert center [42]. All conversions were adjudicated blind to the CSF and plasma biomarker results. In our study, the percentage of conversion was in line with already published studies [50].

The present study has some limitations. Diagnosis of AD was only based on clinical and brain MRI information and not on PIB-PET or on pathological confirmation. Results of CSF biomarkers were not taken into consideration for the AD diagnosis to avoid a circular analysis because of the correlation between CSF and plasma biomarkers. In order to increase the likelihood of conversion to AD we exclude participants with Lewy Body, Parkinson, frontotemporal or vascular MCI disorders. Therefore 77% of subjects had amnesic form of MCI and only 5% of participants converted to a non-Alzheimer's disease. Finally, a large proportion of subject had at least high school diploma, as often observed in such longitudinal, and so the result might not be totally representative a general population.

Conclusion

Our results show the relevance of plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio to identify MCI patients at low risk of conversion to dementia. Using the threshold of 0.169 for the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio, it is possible to determine MCI patients with 48 % less risk to AD conversion, with a good sensitivity. This ratio could be used in clinical practice or in clinical trials to exclude MCI participants that will remain stable over 3 years. Future studies are warranted to validate these promising results.

Bibliography

- [1] Dubois B, Feldman HH, Jacova C, Cummings JL, Dekosky ST, Barberger-Gateau P et al. Revising the definition of Alzheimer's disease: a new lexicon. *Lancet Neurol* 2010;9:1118-27, [https://doi.org/10.1016/S1474-4422\(10\)70223-4](https://doi.org/10.1016/S1474-4422(10)70223-4).
- [2] Dumurgier J, Schraen S, Gabelle A, Vercurysse O, Bombois S, Laplanche J et al. Cerebrospinal fluid amyloid- β 42/40 ratio in clinical setting of memory centers: a multicentric study. *Alzheimers Res Ther* 2015;7:30, <https://doi.org/10.1186/s13195-015-0114-5>.
- [3] Gabelle A, Dumurgier J, Vercurysse O, Paquet C, Bombois S, Laplanche J et al. Impact of the 2008-2012 French Alzheimer Plan on the use of cerebrospinal fluid biomarkers in research memory center: the PLM Study. *J Alzheimers Dis* 2013;34:297-305, <https://doi.org/10.3233/JAD-121549>.
- [4] Lehmann S, Dumurgier J, Schraen S, Wallon D, Blanc F, Magnin E et al. A diagnostic scale for Alzheimer's disease based on cerebrospinal fluid biomarker profiles. *Alzheimers Res Ther* 2014;6:38, <https://doi.org/10.1186/alzrt267>.
- [5] Lehmann S, Delaby C, Boursier G, Catteau C, Ginestet N, Tiers L et al. Relevance of A β 42/40 Ratio for Detection of Alzheimer Disease Pathology in Clinical Routine: The PLM(R) Scale. *Front Aging Neurosci* 2018;10:138, <https://doi.org/10.3389/fnagi.2018.00138>.
- [6] Johnson KA, Sperling RA, Gidicsin CM, Carmasin JS, Maye JE, Coleman RE et al. Florbetapir (F18-AV-45) PET to assess amyloid burden in Alzheimer's disease dementia, mild cognitive impairment, and normal aging. *Alzheimers Dement* 2013;9:S72-83, <https://doi.org/10.1016/j.jalz.2012.10.007>.
- [7] Visser PJ, Knopman DS. Amyloid imaging in the prediction of Alzheimer-type dementia in subjects with amnesic MCI. *Neurology* 2009;73:744-5, <https://doi.org/10.1212/WNL.0b013e3181b61b5d>.
- [8] Koivunen J, Scheinin N, Virta JR, Aalto S, Vahlberg T, Någren K et al. Amyloid PET imaging in patients with mild cognitive impairment: a 2-year follow-up study. *Neurology* 2011;76:1085-90, <https://doi.org/10.1212/WNL.0b013e318212015e>.
- [9] Jack CRJ, Wiste HJ, Vemuri P, Weigand SD, Senjem ML, Zeng G et al. Brain beta-amyloid measures and magnetic resonance imaging atrophy both predict time-to-progression from mild cognitive impairment to Alzheimer's disease. *Brain* 2010;133:3336-48, <https://doi.org/10.1093/brain/awq277>.
- [10] Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med* 2012;367:795-804, <https://doi.org/10.1056/NEJMoa1202753>.
- [11] Teunissen CE, Chiu M, Yang C, Yang S, Scheltens P, Zetterberg H et al. Plasma Amyloid- β (A β 42) Correlates with Cerebrospinal Fluid A β 42 in Alzheimer's Disease. *J Alzheimers Dis* 2018;62:1857-63, <https://doi.org/10.3233/JAD-170784>.
- [12] Hanon O, Vidal J, Lehmann S, Bombois S, Allinquant B, Treluyer J et al. Plasma amyloid levels within the Alzheimer's process and correlations with central biomarkers. *Alzheimers Dement* 2018;14:858-68, <https://doi.org/10.1016/j.jalz.2018.01.004>.
- [13] Risacher SL, Fandos N, Romero J, Sherriff I, Pesini P, Saykin AJ et al. Plasma amyloid beta levels are associated with cerebral amyloid and tau deposition. *Alzheimers Dement (Amst)* 2019;11:510-9, <https://doi.org/10.1016/j.dadm.2019.05.007>.

- [14] Doecke JD, Pérez-Grijalba V, Fandos N, Fowler C, Villemagne VL, Masters CL et al. Total A β (42)/A β (40) ratio in plasma predicts amyloid-PET status, independent of clinical AD diagnosis. *Neurology* 2020;94:e1580-e1591, <https://doi.org/10.1212/WNL.00000000000009240>.
- [15] Lopez OL, Klunk WE, Mathis CA, Snitz BE, Chang Y, Tracy RP et al. Relationship of amyloid- β 1-42 in blood and brain amyloid: Ginkgo Evaluation of Memory Study. *Brain Commun* 2020;2:fcz038, <https://doi.org/10.1093/braincomms/fcz038>.
- [16] Lue L, Sabbagh MN, Chiu M, Jing N, Snyder NL, Schmitz C et al. Plasma Levels of A β 42 and Tau Identified Probable Alzheimer's Dementia: Findings in Two Cohorts. *Front Aging Neurosci* 2017;9:226, <https://doi.org/10.3389/fnagi.2017.00226>.
- [17] Janelidze S, Stomrud E, Palmqvist S, Zetterberg H, van Westen D, Jeromin A et al. Plasma β -amyloid in Alzheimer's disease and vascular disease. *Sci Rep* 2016;6:26801, <https://doi.org/10.1038/srep26801>.
- [18] Verberk IMW, Slot RE, Verfaillie SCJ, Heijst H, Prins ND, van Berckel BNM et al. Plasma Amyloid as Prescreener for the Earliest Alzheimer Pathological Changes. *Ann Neurol* 2018;84:648-58, <https://doi.org/10.1002/ana.25334>.
- [19] Vergallo A, Mégret L, Lista S, Cavedo E, Zetterberg H, Blennow K et al. Plasma amyloid β 40/42 ratio predicts cerebral amyloidosis in cognitively normal individuals at risk for Alzheimer's disease. *Alzheimers Dement* 2019;15:764-75, <https://doi.org/10.1016/j.jalz.2019.03.009>.
- [20] Ovod V, Ramsey KN, Mawuenyega KG, Bollinger JG, Hicks T, Schneider T et al. Amyloid β concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. *Alzheimers Dement* 2017;13:841-9, <https://doi.org/10.1016/j.jalz.2017.06.2266>.
- [21] Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Doré V et al. High performance plasma amyloid- β biomarkers for Alzheimer's disease. *Nature* 2018;554:249-54, <https://doi.org/10.1038/nature25456>.
- [22] Koyama A, Okereke OI, Yang T, Blacker D, Selkoe DJ, Grodstein F. Plasma amyloid- β as a predictor of dementia and cognitive decline: a systematic review and meta-analysis. *Arch Neurol* 2012;69:824-31, <https://doi.org/10.1001/archneurol.2011.1841>.
- [23] van Oijen M, Hofman A, Soares HD, Koudstaal PJ, Breteler MMB. Plasma A β (1-40) and A β (1-42) and the risk of dementia: a prospective case-cohort study. *Lancet Neurol* 2006;5:655-60, [https://doi.org/10.1016/S1474-4422\(06\)70501-4](https://doi.org/10.1016/S1474-4422(06)70501-4).
- [24] de Wolf F, Ghanbari M, Licher S, McRae-McKee K, Gras L, Weverling GJ et al. Plasma tau, neurofilament light chain and amyloid- β levels and risk of dementia; a population-based cohort study. *Brain* 2020;143:1220-32, <https://doi.org/10.1093/brain/awaa054>.
- [25] Lambert J, Schraen-Maschke S, Richard F, Fievet N, Rouaud O, Berr C et al. Association of plasma amyloid β with risk of dementia: the prospective Three-City Study. *Neurology* 2009;73:847-53, <https://doi.org/10.1212/WNL.0b013e3181b78448>.
- [26] Chouraki V, Beiser A, Yountkin L, Preis SR, Weinstein G, Hansson O et al. Plasma amyloid- β and risk of Alzheimer's disease in the Framingham Heart Study. *Alzheimers Dement* 2015;11:249-57.e1, <https://doi.org/10.1016/j.jalz.2014.07.001>.
- [27] Lopez OL, Chang Y, Ives DG, Snitz BE, Fitzpatrick AL, Carlson MC et al. Blood amyloid levels and risk of dementia in the Ginkgo Evaluation of Memory Study (GEMS): A longitudinal analysis. *Alzheimers Dement* 2019;15:1029-38, <https://doi.org/10.1016/j.jalz.2019.04.008>.

- [28] Blasko I, Jellinger K, Kemmler G, Krampla W, Jungwirth S, Wichart I et al. Conversion from cognitive health to mild cognitive impairment and Alzheimer's disease: prediction by plasma amyloid beta 42, medial temporal lobe atrophy and homocysteine. *Neurobiol Aging* 2008;29:1-11, <https://doi.org/10.1016/j.neurobiolaging.2006.09.002>.
- [29] Hansson O, Zetterberg H, Vanmechelen E, Vanderstichele H, Andreasson U, Londos E et al. Evaluation of plasma Aβ(40) and Aβ(42) as predictors of conversion to Alzheimer's disease in patients with mild cognitive impairment. *Neurobiol Aging* 2010;31:357-67, <https://doi.org/10.1016/j.neurobiolaging.2008.03.027>.
- [30] Lewczuk P, Popp J, Lelental N, Kölsch H, Maier W, Kornhuber J et al. Cerebrospinal fluid soluble amyloid-β protein precursor as a potential novel biomarkers of Alzheimer's disease. *J Alzheimers Dis* 2012;28:119-25, <https://doi.org/10.3233/JAD-2011-110857>.
- [31] Alexopoulos P, Tsolakidou A, Roselli F, Arnold A, Grimmer T, Westerteicher C et al. Clinical and neurobiological correlates of soluble amyloid precursor proteins in the cerebrospinal fluid. *Alzheimers Dement* 2012;8:304-11, <https://doi.org/10.1016/j.jalz.2011.04.009>.
- [32] Alexopoulos P, Gleixner L, Werle L, Buhl F, Thierjung N, Giourou E et al. Plasma levels of soluble amyloid precursor protein β in symptomatic Alzheimer's disease. *Eur Arch Psychiatry Clin Neurosci* 2018;268:519-24, <https://doi.org/10.1007/s00406-017-0815-9>.
- [33] Brinkmalm G, Brinkmalm A, Bourgeois P, Persson R, Hansson O, Portelius E et al. Soluble amyloid precursor protein α and β in CSF in Alzheimer's disease. *Brain Res* 2013;1513:117-26, <https://doi.org/10.1016/j.brainres.2013.03.019>.
- [34] Yun S, Cho S, Jo C, Park MH, Han C, Koh YH. Elevation of plasma soluble amyloid precursor protein beta in Alzheimer's disease. *Arch Gerontol Geriatr* 2020;87:103995, <https://doi.org/10.1016/j.archger.2019.103995>.
- [35] Rose C, Dorard E, Audrain M, Gorisse-Hussonnois L, Cartier N, Braudeau J et al. Transient increase in sAPPα secretion in response to Aβ1-42 oligomers: an attempt of neuronal self-defense?. *Neurobiol Aging* 2018;61:23-35, <https://doi.org/10.1016/j.neurobiolaging.2017.09.008>.
- [36] Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol* 1999;56:303-8, <https://doi.org/10.1001/archneur.56.3.303>.
- [37] Portet F, Ousset PJ, Visser PJ, Frisoni GB, Nobili F, Scheltens P et al. Mild cognitive impairment (MCI) in medical practice: a critical review of the concept and new diagnostic procedure. Report of the MCI Working Group of the European Consortium on Alzheimer's Disease. *J Neurol Neurosurg Psychiatry* 2006;77:714-8, <https://doi.org/10.1136/jnnp.2005.085332>.
- [38] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CRJ, Kawas CH et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011;7:263-9, <https://doi.org/10.1016/j.jalz.2011.03.005>.
- [39] Jack CRJ, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement* 2018;14:535-62, <https://doi.org/>.
- [40] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute

on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011;7:270-9, <https://doi.org/>.

- [41] Rose C, Peoc'h K, Chasseigneaux S, Paquet C, Dumurgier J, Bourasset F et al. New highly sensitive rodent and human tests for soluble amyloid precursor protein alpha quantification: preclinical and clinical applications in Alzheimer's disease. *BMC Neurosci* 2012;13:84, <https://doi.org/10.1186/1471-2202-13-84>.
- [42] Operto G, Chupin M, Batrancourt B, Habert M, Colliot O, Benali H et al. CATI: A Large Distributed Infrastructure for the Neuroimaging of Cohorts. *Neuroinformatics* 2016;14:253-64, <https://doi.org/10.1007/s12021-016-9295-8>.
- [43] Pencina MJ, D'Agostino RB, Pencina KM, Janssens ACJW, Greenland P. Interpreting incremental value of markers added to risk prediction models. *Am J Epidemiol* 2012;176:473-81, <https://doi.org/10.1093/aje/kws207>.
- [44] Fei M, Jianghua W, Rujuan M, Wei Z, Qian W. The relationship of plasma A β levels to dementia in aging individuals with mild cognitive impairment. *J Neurol Sci* 2011;305:92-6, <https://doi.org/10.1016/j.jns.2011.03.005>.
- [45] Cammarata S, Borghi R, Giliberto L, Pardini M, Pollero V, Novello C et al. Amyloid-beta42 plasma levels are elevated in amnesic mild cognitive impairment. *J Alzheimers Dis* 2009;18:267-71, <https://doi.org/10.3233/JAD-2009-1144>.
- [46] Lopez OL, Kuller LH, Mehta PD, Becker JT, Gach HM, Sweet RA et al. Plasma amyloid levels and the risk of AD in normal subjects in the Cardiovascular Health Study. *Neurology* 2008;70:1664-71, <https://doi.org/10.1212/01.wnl.0000306696.82017.66>.
- [47] Blennow K, De Meyer G, Hansson O, Minthon L, Wallin A, Zetterberg H et al. Evolution of Abeta42 and Abeta40 levels and Abeta42/Abeta40 ratio in plasma during progression of Alzheimer's disease: a multicenter assessment. *J Nutr Health Aging* 2009;13:205-8, <https://doi.org/10.1007/s12603-009-0059-0>.
- [48] Lin Z, Sur S, Soldan A, Pettigrew C, Miller M, Oishi K et al. Brain Oxygen Extraction by Using MRI in Older Individuals: Relationship to Apolipoprotein E Genotype and Amyloid Burden. *Radiology* 2019;292:140-8, <https://doi.org/10.1148/radiol.2019182726>.
- [49] Lachno DR, Emerson JK, Vanderstichele H, Gonzales C, Martenyi F, Konrad RJ et al. Validation of a multiplex assay for simultaneous quantification of amyloid- β peptide species in human plasma with utility for measurements in studies of Alzheimer's disease therapeutics. *J Alzheimers Dis* 2012;32:905-18, <https://doi.org/10.3233/JAD-2012-121075>.
- [50] Mitchell AJ, Shiri-Feshki M. Rate of progression of mild cognitive impairment to dementia--meta-analysis of 41 robust inception cohort studies. *Acta Psychiatr Scand* 2009;119:252-65, <https://doi.org/10.1111/j.1600-0447.2008.01326.x>.

Table 1. Characteristics in the whole MCI population and between MCI that converted or not to dementia within 3 years.

| General characteristics, % (N) | MCI sample N=485 | Conversion to dementia | | P value* | P value† |
|--|---------------------|------------------------|-------------|----------|----------|
| | | Yes N=145 | No N=340 | | |
| Age (years), M(SD) | 77.7 (5.5) | 78.6 (5.7) | 77.4 (5.4) | 0.03 | 0.002 |
| Woman | 60.4 (293) | 60.7 (88) | 60.3 (205) | 0.99 | 0.97 |
| Education level | | | | | |
| Primary | 15.7 (76) | 15.9 (23) | 15.6 (53) | | |
| Secondary | 36.4 (176) | 36.6 (53) | 36.3 (123) | 0.99 | 0.97 |
| High school diploma or above | 47.9 (232) | 47.6 (69) | 48.1 (163) | | |
| Tobacco | | | | | |
| Current | 7.4 (34) | 5.8 (8) | 8.12 (26) | | |
| Former | 60.6 (277) | 62.0 (85) | 60.0 (192) | 0.69 | 0.87 |
| Never | 31.9 (146) | 32.1 (44) | 31.9 (102) | | |
| BMI, M(SD) | 25.0 (3.8) | 24.8 (3.8) | 25.1 (3.8) | 0.37 | 0.94 |
| Comorbidity | | | | | |
| Hypertension | 71.8 (346) | 75.0 (108) | 70.4 (238) | 0.36 | 0.25 |
| Mellitus Diabetes | 15.0 (68) | 12.4 (17) | 16.2 (51) | 0.37 | 0.44 |
| Dyslipidemia | 38.4 (184) | 34.0 (49) | 40.3 (135) | 0.23 | 0.13 |
| History of stroke or TIA | 6.8 (33) | 6.2 (9) | 7.1 (24) | 0.85 | 0.83 |
| History of depression | 24.6 (118) | 20.3 (29) | 26.5 (89) | 0.18 | 0.28 |
| Global cognitive assessment, M(SD) | | | | | |
| MMSE (/30) | 26.4 (2.5) | 25.6 (2.5) | 26.8 (2.5) | <.0001 | 0.0001 |
| ADL score (/6) | 5.8 (0.38) | 5.9 (0.32) | 5.8 (0.40) | 0.45 | 0.46 |
| IADL score (/14) | 12.8 (1.9) | 12.6 (1.9) | 12.9 (1.9) | 0.20 | 0.42 |
| GDS (/30) | 8.0 (5.17) | 7.9 (5.06) | 8.1 (5.22) | 0.65 | 0.99 |
| Amnesic MCI | 76.9 (373) | 92.4 (134) | 70.3 (239) | <.0001 | <.0001 |
| APOE ε4 carrier, %(N) | 39.6 (191) | 56.6 (82) | 32.3 (109) | <.0001 | <.0001 |
| Hippocampal atrophy (Scheltens scale) | | | | | |
| None (0 or 1 on both sides) | 43.8 (172) | 26.9 (32) | 51.1 (140) | | |
| Moderate (none of the others) | 28.2 (111) | 28.6 (34) | 28.1 (77) | <.0001 | <.0001 |
| Severe (3 or 4 on at least 1 side) | 28.0 (110) | 44.5 (53) | 20.8 (57) | | |

*, p-values from parametric tests (ANOVA or χ^2)

†, p-values from multinomial logistic regression adjusted for age, sex, education level, and APOE ε4

Abbreviations: MCI, mild cognitive impairment; TIA, transient ischemic attack; BMI, body mass index in kg/m²; MMSE, mini mental state examination; ADL, activities of daily living; IADL, instrumental activities of daily living; GDS, geriatric depression scale

Table 2. Plasma and CSF biomarkers in the whole MCI population and between MCI that converted or not to dementia within 3 years.

| Biomarkers M (SD) | MCI sample | Conversion to dementia | | P value* | P value† |
|--|---------------|------------------------|---------------|----------|----------|
| | | Yes | No | | |
| Plasma/serum biomarkers | N=485 | N=145 | N=340 | | |
| A β ₁₋₄₂ (pg/mL) | 38.5 (11.6) | 37.1 (12.5) | 39.2 (11.1) | 0.03 | 0.04 |
| A β ₁₋₄₀ (pg/mL) | 270 (65) | 275 (73) | 268 (61) | 0.71 | 0.80 |
| sAPP α (ng/mL) | 466 (170) | 458 (149) | 470 (179) | 0.76 | 0.82 |
| A β ₁₋₄₂ /A β ₁₋₄₀ ratio | 0.153 (0.094) | 0.148 (0.125) | 0.154 (0.076) | 0.02 | 0.04 |
| A β ₁₋₄₂ /A β ₁₋₄₀ ratio quartiles, %(N) | | | | | |
| Q1 [0.033;0.119] | 25.2 (122) | 32.4 (47) | 22.1 (75) | | |
| Q2 (0.119;0.144] | 24.9 (121) | 24.8 (36) | 25.0 (85) | 0.005 | 0.02 |
| Q3 (0.144;0.169] | 24.9 (121) | 27.6 (40) | 23.8 (81) | | |
| Q4 (0.169;1.5] | 24.9 (121) | 15.2 (22) | 29.1 (99) | | |
| CSF biomarkers | N=228 | N=50 | N=178 | | |
| A β ₁₋₄₂ (pg/mL) | 906 (391) | 716 (290) | 999 (402) | <.0001 | <.0001 |
| Tau (pg/mL) | 433 (213) | 546 (222) | 381 (188) | <.0001 | <.0001 |
| p-Tau (pg/mL) | 65.8 (29.3) | 80.0 (33.5) | 58.8 (24.2) | <.0001 | <.0001 |
| sAPP α (ng/mL) | 623 (219) | 637 (207) | 617 (225) | 0.29 | 0.30 |
| A β ₁₋₄₂ /p-Tau ratio | 16.9 (10.5) | 10.9 (6.8) | 19.9 (10.7) | <.0001 | <.0001 |
| PLM scale, %(N) | | | | | |
| 0 | 31.6 (72) | 11.1 (8) | 41.0 (64) | | |
| 1 | 30.3 (69) | 26.4 (19) | 32.1 (50) | <.0001 | <.0001 |
| 2 | 10.5 (24) | 11.1 (8) | 10.3 (16) | | |
| 3 | 27.6 (63) | 51.4 (37) | 16.7 (26) | | |
| PLM scale 3, %(N) | 27.6 (63) | 51.4 (37) | 16.7 (26) | <.0001 | <.0001 |
| MCI classification | | | | | |
| Core clinical | 57.3 (278) | 44.1 (64) | 62.9 (214) | | |
| High likelihood | 18.8 (91) | 34.5 (50) | 12.1 (41) | <.0001 | <.0001 |
| Intermediate likelihood | 12.8 (62) | 20.7 (30) | 9.4 (32) | | |
| Low likelihood | 11.1 (54) | 0.6 (1) | 15.6 (53) | | |

*, p-values from parametric tests (ANOVA or χ^2)

†, p-values from multinomial logistic regression adjusted for age, sex, education level, and APOE $\epsilon 4$

Abbreviations: MCI, mild cognitive impairment; CSF, cerebrospinal fluid; The PLM scale counts the number of altered biomarkers (A β ₁₋₄₂, Tau, p-Tau): PLM0 corresponds to A-T-N-; PLM1 and PLM2 indicate the presence of 1 or 2 positive biomarkers respectively, whatever the biomarker; PLM3 corresponds to A+T+N+

Table 3. Characteristics and plasma and CSF biomarkers in MCI participants in the highest quartile vs. 3 lower quartiles of $A\beta_{1-42}/A\beta_{1-40}$ ratio.

| Characteristics, % (N) | $A\beta_{1-42}/A\beta_{1-40}$ ratio | | P value* | P value† |
|--|-------------------------------------|----------------------------|----------|----------|
| | Highest quartile N=122 | 3 lower quartiles N=363 | | |
| Age (years), M (SD) | 78.3 (5.4) | 77.5 (5.5) | 0.16 | 0.37 |
| Woman | 63.1 (77) | 59.5 (216) | 0.55 | 0.75 |
| Education level | | | | |
| Primary | 15.6 (19) | 15.7 (57) | | |
| Secondary | 43.4 (53) | 34.0 (123) | 0.14 | 0.15 |
| High school diploma or above | 41.0 (50) | 50.3 (182) | | |
| Tobacco | | | | |
| Current | 5.26 (6) | 8.16 (28) | | |
| Former | 63.2 (72) | 59.8 (205) | 0.57 | 0.54 |
| Never | 31.6 (36) | 32.1 (110) | | |
| BMI, M (SD) | 25.2 (3.7) | 25.0 (3.8) | 0.67 | 0.79 |
| Conversion MCI to dementia | 18.0 (22) | 33.9 (123) | 0.001 | 0.002 |
| Comorbidity | 21.7 (26) | 30.4 (110) | | |
| Hypertension | 78.3 (94) | 69.6 (252) | 0.08 | 0.13 |
| Mellitus Diabetes | 17.1 (18) | 14.4 (50) | 0.6 | 0.43 |
| Dyslipidemia | 37.7 (46) | 38.7 (138) | 0.94 | 0.97 |
| History of stroke or TIA | 4.92 (6) | 7.54 (27) | 0.43 | 0.26 |
| History of depression | 21.3 (26) | 25.8 (92) | 0.39 | 0.28 |
| Global cognitive assessment, M (SD) | | | | |
| MMSE (/30) | 26.5 (2.9) | 26.4 (2.4) | 0.53 | 0.53 |
| ADL score (/6) | 5.90 (0.27) | 5.88 (0.41) | 0.57 | 0.42 |
| IADL score (/14) | 12.8 (1.6) | 12.8 (2.0) | 0.72 | 0.63 |
| GDS (/30) | 8.65 (5.39) | 7.89 (5.09) | 0.18 | 0.31 |
| Amnesic MCI | 73.0 (89) | 78.2 (284) | 0.28 | 0.38 |
| APOE ϵ4 carrier | 29.2 (35) | 43.1 (156) | 0.009 | 0.01 |
| Hippocampal atrophy (Scheltens scale) | | | | |
| None (0 or 1 on both sides) | 44.3 (43) | 43.6 (129) | | |
| Moderate (none of the others) | 27.8 (27) | 28.4 (84) | 0.99 | 0.91 |
| Severe (3 or 4 on at least 1 side) | 27.8 (27) | 28.0 (83) | | |
| Plasma/serum biomarkers, M (SD) | | | | |
| $A\beta_{1-42}$ (pg/mL) | 46.2 (11.3) | 36.0 (10.5) | <.0001 | <.0001 |
| $A\beta_{1-40}$ (pg/mL) | 236 (73) | 282 (58) | <.0001 | <.0001 |
| sAPP α (ng/mL) | 490 (185) | 459 (165) | 0.1 | 0.12 |
| CSF biomarkers, M (SD) | N=50 | N=178 | | |
| $A\beta_{1-42}$ (pg/mL) | 1025 (403) | 873 (383) | 0.01 | 0.04 |
| Tau (pg/mL) | 353 (152) | 455 (223) | 0.003 | 0.006 |
| p-Tau (pg/mL) | 53.7 (16.0) | 69.1 (31.2) | 0.0009 | 0.002 |
| sAPP α (ng/mL) | 580 (205) | 635 (222) | 0.15 | 0.11 |
| $A\beta_{1-42}/p$ -Tau ratio | 20.7 (9.9) | 15.9 (10.4) | 0.004 | 0.008 |
| PLM scale | | | | |
| 0 | 38.0 (19) | 29.8 (53) | | |
| 1 | 40.0 (20) | 27.5 (49) | | |
| 2 | 16.0 (8) | 8.99 (16) | 0.001 | 0.0006 |
| 3 | 6.00 (3) | 33.7 (60) | | |
| PLM scale 3 | 6.00 (3) | 33.7 (60) | 0.0002 | <.0001 |
| MCI classification | | | | |
| Core clinical | 68.0 (83) | 53.7 (195) | | |
| High likelihood | 8.2 (10) | 22.3 (81) | 0.005 | 0.006 |
| Intermediate likelihood | 13.1 (16) | 12.7 (46) | | |
| Low likelihood | 10.7 (13) | 11.3 (41) | | |

*, p-values from parametric tests (ANOVA or χ^2)

†, p-values from multinomial logistic regression adjusted for age, sex, education level, and APOE ϵ 4

Abbreviations: MCI, mild cognitive impairment; TIA, transient ischemic attack; BMI, body mass index in kg/m^2 ; MMSE, mini mental state examination; ADL, activities of daily living; IADL, instrumental

activities of daily living; GDS, geriatric depression scale; CSF, cerebrospinal fluid; The PLM scale counts the number of altered biomarkers ($A\beta_{1-42}$, Tau, p-Tau): PLM0 corresponds to A-T-N-; PLM1 and PLM2 indicate the presence of 1 or 2 positive biomarkers respectively, whatever the biomarker; PLM3 corresponds to A+T+N+.

Table 4. Multivariate analysis of conversion and A β ₁₋₄₂/A β ₁₋₄₀ ratio with Cox and logistic models

| | Cox model | | Logistic model | |
|--|------------------|----------------|------------------|----------------|
| | HR (95% CI) | <i>P</i> value | OR (95% CI) | <i>P</i> value |
| A β ₁₋₄₂ /A β ₁₋₄₀ ratio Q4 vs. Q123 | 0.52 (0.31-0.86) | 0.01 | 0.45 (0.23-0.82) | 0.01 |
| Age | 1.03 (1.00-1.07) | 0.06 | 1.04 (0.99-1.09) | 0.10 |
| Women | 1.37 (0.92-2.04) | 0.12 | 1.50 (0.90-2.53) | 0.12 |
| Education (primary as reference class) | | | | |
| Secondary | 0.94 (0.53-1.65) | 0.83 | 1.27 (0.62-2.66) | 0.62 |
| High school diploma and above | 0.97 (0.55-1.73) | 0.92 | 1.20 (0.59-2.54) | 0.52 |
| <i>APOE</i> ϵ 4 carriers | 1.98 (1.36-2.89) | 0.0004 | 2.59 (1.58-4.29) | 0.0002 |
| MMSE at baseline | 0.87 (0.81-0.93) | <.0001 | 0.87 (0.79-0.96) | 0.006 |
| Hippocampus atrophy (Scheltens scale) | | | | |
| Moderate atrophy | 1.59 (0.97-2.62) | 0.07 | 1.78 (0.97-3.27) | 0.06 |
| Severe atrophy | 3.04 (1.89-4.91) | <.0001 | 4.07 (2.22-7.59) | <.0001 |

Abbreviations: HR, hazard ratio; OR, odds ratio; Q, quartile; A β , amyloid β ; MMSE, mini mental state evaluation; *APOE*, apolipoprotein epsilon.

Table 5. Improvement of MCI conversion to dementia risk with $A\beta_{1-42}/A\beta_{1-40}$ ratio

| | $A\beta_{1-42}/A\beta_{1-40}$ ratio | $A\beta_{1-42}/A\beta_{1-40}$ ratio |
|--------------|--|--|
| | Continuous | Higher quartile vs. 3 lower quartiles |
| IDI [95% CI] | 0.0004 (-0.001; 0.002), <i>P</i> value =0.56 | 0.018 (0.007; 0.030), <i>P</i> value =0.0009 |
| NRI [95% CI] | 0.221 (0.033; 0.409), <i>P</i> value =0.02 | 0.274 (0.122; 0.426), <i>P</i> value =0.0004 |

Abbreviations: $A\beta$, amyloid β ; IDI, integrated discrimination improvement; NRI, net reclassification improvement.

Figure 1. Kaplan Meier curve of conversion to dementia according to the quartiles of plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio

Abbreviations: MCI, mild cognitive impairment; $A\beta$, amyloid β ;

Acknowledgments

Conflicts of interest: none

Funding Sources

BALTAZAR study is supported by 2 grants from the French ministry of Health (Programme Hospitalier de Recherche Clinique (PHRC 2009 / 01-04 and PHRC-13-0404)) and by the Fondation Plan Alzheimer.

Collaborators

The BALTAZAR study group: Olivier Hanon [1], Frédéric Blanc [2], Yasmina Boudali [1], Audrey Gabelle [3], Jacques Touchon [3], Marie-Laure Seux [1], Hermine Lenoir [1], Catherine Bayle [1], Stéphanie Bombois [4], Christine Delmaire [4], Xavier Delbeuck [5], Florence Moulin [1], Emmanuelle Duron [6], Florence Latour [7], Matthieu Plichart [1], Sophie Pichierri [8], Galdric Orvoën [1], Evelyne Galbrun [9], Giovanni Castelnovo [10], Lisette Volpe-Gillot [11], Florian Labourée [1], Pascaline Cassagnaud [12], Claire Paquet [13], Françoise Lala [14], Bruno Vellas [14], Julien Dumurgier [13], Anne-Sophie Rigaud [1], Christine Perret-Guillaume [15], Eliana Alonso [16], Foucaud du Boisgueheneuc [17], Laurence Hugonot-Diener [1], Adeline Rollin-Sillaire [12], Olivier Martinaud [18], Clémence Bouilly [1], Yann Spivac [19], Agnès Devendeville [20], Joël Belmin [21], Philippe Robert [22], Thierry Dantoine [23], Laure Caillard [1], David Wallon [24], Didier Hannequin [18], Nathalie Sastre [14], Sophie Haffen [25], Anna Kearney-Schwartz [15], Jean-Luc Novella [26], Vincent Deramecourt [12], Valérie Chauvire [27], Gabiel Abitbol [1], Nathalie Schwald [19], Caroline Hommet [28], François Sellal [29], Marie-Ange Cariot [16], Mohamed Abdellaoui [30], Sarah Benisty [31], Salim Gherabli [1], Pierre Anthony [29], Frédéric Bloch [32], Nathalie Charasz [1], Sophie Chauvelier [1], Jean-Yves Gaubert [1], Guillaume Sacco [22], Olivier Guerin [22], Jacques Boddaert [33], Marc Paccalin [17], Marie-Anne Mackowiak [12], Marie-Thérèse Rabus [9], Valérie Gissot [34], Athanase Benetos [15], Candice Picard [20], Céline Guillemaud [35], Gilles Berrut [8], Claire Gervais [22], Jaques Hugon [13], Jean-Marc Michel [29], JeanPhilippe David [19], Marion Paulin [12], Pierre-Jean Ousset [14], Pierre Vandel [36], Sylvie Pariel [21], Vincent Camus [37], Anne Chawakilian [1], Léna Kermanac'h [1], Anne-Cécile Troussiere [12], Cécile Adam [23], Diane Dupuy [20], Elena Paillaud [16], Héléne Briault [9], Isabelle Saulnier [38], Karl Mondon [37], Marie-Agnès Picat [23], Marie Laurent [16], Olivier Godefroy [20], Rezki Daheb [16], Stéphanie Libercier [29], Djamilia Krabchi [1], Marie Chupin [39], JeanSébastien Vidal [1], Edouard Chaussade [1], Christiane Baret-Rose [40], Sylvain Lehmann [41], Bernadette Allinquant [40], Susanna Schraen-Maschke [4].

Affiliations

[1] Université de Paris, EA 4468, APHP, Hopital Broca, Memory Resource and Research Centre of de Paris-Broca-Ile de France, F-75013 Paris, France.

[2] Université de Strasbourg, Hôpitaux Universitaires de Strasbourg, CM2R, pôle de Gériatrie, Laboratoire ICube, FMTS, CNRS, équipe IMIS, F-67000 Strasbourg, France.

[3] Université de Montpellier, CHU Montpellier, Memory Research and Resources center of Montpellier, department of Neurology, Inserm INM NeuroPEPs team, excellence center of neurodegenerative disorders, F-34000 Montpellier, France.

[4] Univ. Lille, Inserm, CHU Lille, U1172-LiINCog, LiCEND, LabEx DISTALZ, F-59000 Lille, France.

[5] Univ. Lille, Inserm U1171 Degenerative and Vascular Cognitive Disorders, F-59000 Lille, France.

[6] Université Paris-Saclay, APHP, Hôpital Paul Brousse, département de gériatrie, Équipe MOODS, Inserm 1178, F-94800 Villejuif, France.

[7] Centre Hospitalier de la Côte Basque, Department of Gerontology, F-64100 Bayonne, France.

[8] Université de Nantes, EA 4334 Movement-Interactions-Performance, CHU Nantes, Memory Research Resource Center of Nantes, Department of clinical gerontology, F-44000 Nantes, France.

- [9] Sorbonne Université, APHP, Centre Hospitalier Dupuytren, Department of Gérontology 2, F-91210 Draveil, France.
- [10] CHU de Nîmes, Hôpital Caremeau, Neurology Department, F-30029 Nîmes, France.
- [11] Hôpital Léopold Bellan, Service de Neuro-Psycho-Gériatrie, Memory Clinic, F-75014 Paris, France.
- [12] Univ. Lille, CHU de Lille, Memory Resource and Research Centre of Lille, Department of Neurology, F-59000 Lille, France.
- [13] Université de Paris, APHP, Groupe Hospitalier Saint Louis-Lariboisière-Fernand Widal, Center of Cognitive Neurology, F-75010 Paris, France.
- [14] Université de Toulouse III, CHU La Grave-Casselardit, Memory Resource and Research Centre of Midi-Pyrénées, F-31300 Toulouse, France.
- [15] Université de Lorraine, CHRU de Nancy, Memory Resource and Research Centre of Lorraine, F-54500 Vandœuvre-lès-Nancy, France.
- [16] Université de Paris, APHP, Hôpital européen Georges Pompidou, Service de Gériatrie, F-75015, Paris, France.
- [17] CHU de Poitiers, Memory Resource and Research Centre of Poitiers, F-86000 Poitiers, France.
- [18] CHU Charles Nicolle, Memory Resource and Research Centre of HauteNormandie, F-76000 Rouen, France.
- [19] APHP, Centre Hospitalier Émile-Roux, Department of Gérontology 1, F-94450 Limeil-Brévannes, France.
- [20] CHU d'Amiens-Picardie, Memory Resource and Research Centre of AmiensPicardie, F-80000 Amiens, France.
- [21] Sorbonne Université, APHP, Hôpitaux Universitaires Pitie-Salpêtrière-Charles Foix, Service de Gériatrie Ambulatoire, F-75013 Paris, France.
- [22] Université Côte d'Azur, CHU de Nice, Memory Research Resource Center of Nice, CoBTek lab, F-06100 Nice, France.
- [23] CHU de Limoges, Memory Research Resource Center of Limoges, F-87000 Limoges, France.
- [24] Normandie Univ, UNIROUEN, Inserm U1245, CHU de Rouen, Department of Neurology and CNR-MAJ, Normandy Center for Genomic and Personalized Medicine, CIC-CRB1404, F-76000, Rouen, France.
- [25] CHU de Besançon, Memory Resource and Research Centre of BesançonFranche-Comté, F-25000 Besançon, France.
- [26] Université de Reims Champagne-Ardenne, EA 3797, CHU de Reims, Memory Resource and Research Centre of Champagne-Ardenne, F-51100 Reims, France.
- [27] CHU d'Angers, Memory Resource and Research Centre of Angers, F-49000 Angers, France.
- [28] CHRU de Tours, Memory Resource and Research Centre of Tours, F-37000 Tours, France.
- [29] Université de Strasbourg, CHRU de Strasbourg, Memory Resource and Research Centre of Strasbourg/Colmar, Inserm U-118, F-67000 Strasbourg, France.

- [30] Univ Paris Est Creteil, EA 4391 Excitabilité Nerveuse et Thérapeutique, CHU Henri Mondor, Department of Neurology, F-94000 Créteil, France.
- [31] Hôpital Fondation Rothschild, Department of Neurology, F-75019 Paris, France.
- [32] CHU d'Amiens-Picardie, Department of Gerontology, F-80000 Amiens, France.
- [33] Sorbonne Université, APHP, Hôpitaux Universitaires Pitie-Salpêtrière-Charles Foix, Memory Resource and Research Centre, Centre des Maladies Cognitives et Comportementales IM2A, Inserm UMR 8256, F-75013 Paris, France.
- [34] Université François-Rabelais de Tours, CHRU de Tours, Memory Resource and Research Centre of Tours, Inserm CIC 1415, F-37000 Tours, France.
- [35] Sorbonne Université, APHP, Hôpitaux Universitaires Pitie-Salpêtrière-Charles Foix, Memory Resource and Research Centre, Centre des Maladies Cognitives et Comportementales IM2A, F-75013 Paris, France.
- [36] Université Bourgogne Franche-Comté, Laboratoire de Recherches Intégratives en Neurosciences et Psychologie Cognitive, CHU de Besançon, Memory Resource and Research Centre of Besançon Franche-Comté, F-25000 Besançon, France.
- [37] Université François-Rabelais de Tours, CHRU de Tours, UMR Inserm U1253, F-37000 Tours, France.
- [38] Université de Limoges, EA 6310 HAVAE, CHU de Limoges, Memory Research Resource Center of Limoges, F-87000 Limoges, France.
- [39] Université Paris-Saclay, Neurospin, CEA, CNRS, cati-neuroimaging.com, CATI Multicenter Neuroimaging Platform, F-91190 Gif-sur-Yvette, France.
- [40] Université de Paris, Institute of Psychiatric and Neurosciences, Inserm UMR-S 1266, F-75014 Paris, France.
- [41] Université de Montpellier, CHU Montpellier, LBPC, Inserm, F-34000 Montpellier, France.