Prognostic value of Alzheimer's biomarkers in mild cognitive impairment: the effect of age at onset

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ABSTRACT

Objective. The aim of this study is to assess the impact of age at onset on the prognostic value of Alzheimer's biomarkers in a large sample of patients with mild cognitive impairment (MCI).

Methods. We measured Aβ42, t-tau, hippocampal volume on magnetic resonance imaging (MRI) and cortical metabolism on fluorodeoxyglucose-positron emission tomography (FDG-PET) in 188 MCI patients followed for at least 1 year. We categorised patients into earlier and later onset (EO/LO). Receiver operating characteristic curves and corresponding areas under the curve (AUCs) were performed to assess and compar the biomarker prognostic performances in EO and LO groups. Linear Model were adopted for estimating the time-to-progression in relation with earlier/later onset MCI groups and biomarkers.

Results. In earlier onset patients, all the assessed biomarkers were able to predict cognitive decline ($p<0.05$), with FDG-PET showing the best performance. In later onset patients, all biomarkers but t-tau predicted cognitive decline ($p<0.05$). Moreover, FDG-PET alone in earlier onset patients showed a higher prognostic value than the one resulting from the combination of all the biomarkers in later onset patients (earlier onset AUC=0.935 vs later onset AUC=0.753, $p<0.001$). Finally, FDG-PET showed a different prognostic value between earlier and later onset patients ($p=0.040$) in time-to-progression allowing an estimate of the time free from disease.

Discussion. FDG-PET may represent the most universal tool for the establishment of a prognosis in MCI patients and may be used for obtaining an onset-related estimate of the time free from disease.

KEYWORDS

Alzheimer, Cognition, Imaging, Biomarkers, FDG-PET

1. INTRODUCTION

Diagnostic criteria for Alzheimer's disease [1, 2] postulate that the assessment of diagnostic/pathophysiological (amyloid and tau pathology) and progression/topographical (cortical atrophy and hypometabolism) biomarkers enhances the diagnostic accuracy. Although a large amount of data supported the accuracy of these biomarkers for the diagnosis of Alzheimer's disease at both the dementia [3–5] and the mild cognitive impairment (MCI) stages [4, 6, 7], only relative few studies compared biomarkers in terms of diagnostic and prognostic performances. FDG-PET alone showed a prognostic performance comparable to CSF Aβ42 paired with hippocampal atrophy [8], while the joint use of MRI, FDG-PET, and CSF biomarkers provided the most accurate prediction of dementia [9–11]. Covariates associated with the disease, such as age at symptom onset, may affect biomarker status or level, and thus its diagnostic or prognostic performance. Indeed, younger age at onset was associated with milder hippocampal atrophy [12, 13], and greater amyloid burden [14] and parietal hypometabolism [15]. Thus, MRI exhibited higher diagnostic value for later onset [16] while FDG-PET [17] and Alzheimer's CSF biomarkers [18] showed best performance in earlier onset patients with Alzheimer's disease. However most of these works, as well as recent papers, in which the age of onset and biomarkers level have been concurrently assessed in MCI, include relative small samples of patients and often with different cognitive profile [19–22], thus a validation on a large, honogeneous sample of amnestic MCI is desirable. A noteworthy aspect regards the quantification of biomarker ability to predict the time-to-progression. Very few studies compare biomarkers in terms of quantification of time-to-conversion [23] and, to the best of our knowledge, none faced with quantification of the effect of age at onset on the biomarker ability to estimate time-to-progression. The aim of this study was to investigate the impact of age at symptom onset on the prognostic value of Alzheimer's core biomarkers with respect to cognitive decline in a large sample of MCI patients homogeneous in terms of cognitive profile and impairment severity. Moreover, based on age of onset and biomarkers level, prediction models for the estimate of the time-to-progression was developed.

2. MATERIAL AND METHODS

2.1 Subjects

We selected patients from two independent datasets: the Alzheimer's Disease Neuroimaging Initiative (ADNI, adni.loni.usc.edu) and a harmonised European dataset (EU) of patients coming to diagnostic work-up at four independent European memory clinics (TOMC, Brescia, Italy; VUmc, Amsterdam, The Netherlands; KUHH, Stockholm, Sweden; and TUM, Munich, Germany). For a complete description of the two datasets please see online supplementary materials.

At baseline, all patients enrolled in this study (89 from ADNI, 31 from TOMC, 25 from VUmc, 17 from KUHH and 26 from TUM) were diagnosed as MCI as described by Petersen and colleagues [24], and had data on MRI, FDG-PET and CSF sampling. Biomarker status was either not available or not taken into account for the baseline diagnosis. The cognitive profile was consistent with single or multiple domain amnestic MCI. No focal ischemic lesions nor extensive microvascular disease that could be responsible for the cognitive symptoms were present on routine MR. MCI patients were followed up to detect cognitive decline, defined as (i) having dementia at follow up, ii) getting a score less than 24 at last MMSE (this cut-off showed 66% sensitivity and 99% specificity in detecting dementia; [25], and (iii) losing more than 3 points between first and last MMSE adminstration [26]. In the case a diagnosis of incident dementia was made, only those patients diagnosed as Alzheimer's disease according to NINCDS-ADRDA criteria [27] were included in the study, while MCI patients who converted to non-Alzheimer's dementia were excluded.

2.2 Standard protocol approvals, registrations, and patient consents

Ethics/radiation committee approval of any protocol involved in the study was obtained at each centre. Written informed consent to share data for scientific research purposes was collected from each participant.

2.3 Biological markers of amyloidosis and neurodegeneration

We selected CSF Aβ42 concentration as a biological marker for the absence or presence of cortical amyloid deposition. We assessed neurodegeneration using: i) CSF total tau (t-tau) concentration, ii) an FDG-PET index of Alzheimer-related hypometabolism, the 'AD t-sum' score, calculated using the PMOD Alzheimer's Analysis for FDG PET (PALZ) tool ([28]; http://www.pmod.com), and iii) an MRI-based automated segmentation of age-adjusted hippocampal atrophy (W-scores). Information on procedures, normalization and definition of normality/abnormality thresholds for the biomarkers are reported in online supplementary materials.

2.4 Statistical analysis

We pooled MCI patients from ADNI and EU datasets, and then categorized them into earlier onset (EO, ≤70 years) and later onset (LO, >70 years) based on their age at diagnosis, under the assumption that the arrive to a memory clinic for cognitive impairment corresponds to the symptom onset. Seventy years cut-off was chosen based on: i) recent revisions

of the definition of elderly in Western countries [29–31] and ii) age distribution of our sample (mean=71, median=73). This cut-off allowed to obtain two almost size-balanced groups (40% in EO and 60% il LO). Moreover, in order to validate our results, we repeated all analyses (see online supplementary materials) re-categorising patients into EO and LO by using the conventional 65 years cut-off[32]. One outlier (in EO) was identified by Tukey's Inter-Quantile Range and excluded from the analyses.

We assessed differences between EO and LO in sociodemographic and clinical features, genotype and biomarker statur and leve by Mann-Whitney test (for continuous variables) or Pearson's Chi-squared test (for dichotomous variables). In order to assess the association of biomarker abnormality with cognitive decline, for each biomarker we divided the whole MCI group, as well as EO and LO groups, into subgroups based on biomarker abnormality thresholds [33]. We performed survival curves and univariate Cox regression models (both crude and adjusted by age, gender, APOE, MMSE scores, and study center) individually for each of the 4 biomarkers in the EO, LO and whole MCI groups. Significance of differences in survival curves between normal and abnormal groups were assessed by Log Rank test. Hazard ratios and corresponding 95% confidence intervals (95% CI) were reported for each biomarker evaluated through Cox models.

Receiver operating characteristic (ROC) curves and corresponding areas under the curve (AUCs) were performed to assess and compar the biomarker prognostic performances in EO, LO and whole MCI groups. Within biomarker, EO and LO AUCs were compared using De Long test for two uncorrelated ROC curves [34]. Within patient group, different biomarkers AUCs were compared two by two using the Bootstrap test for two correlated ROC curves. Moreover, in order to take into account differences of follow-up time across patients, time-dependent ROC curves were performed.

A composite evaluation of all biomarkers significantly related to the cognitive decline was carried out by multiple Cox regression models in EO and LO patients (two separate models). Biomarkers were included in the models as continuous variables, and FDG-PET AD t-sum scores were polarized so that more negative values denote greater abnormality. A compound evaluation of biomarkers prognotic capability in detecting cognitive decline (for EO and LO) was obtained by the *risk scores* computed as the predicted values [35] of the Cox regression model, in which only significant biomarkers were included (EO: FDG-PET only; LO: FDG-PET, hippocampal volume, and CSF Aβ). We finally assessed and compared the prognostic performance, evaluated by predicted risk scores, of EO and LO through ROC curves, computing areas under the curve (AUCs). De Long test was used to compare the two (for EO and LO) uncorrelated ROC curves.

Finally, we adopted a Linear Model for analysing the time-to-progression in EO and LO MCI patients, where the time-to progression (in months) was the dependent variable, whereas group (EO/LO, reference category: EO) and

respectively CSF Aβ42, CSF t-tau, FDG-PET, hippocampal volume were independent variables of the three Linear models. Parameters were computed both in the unadjusted model and adjusting by covariates found significant in the Cox models. In case the interaction between biomarker and group was not significant, the interaction was removed from the model. All the above analyses were performed separately also in EU and ADNI cohorts.

We performed statistical analyses using the R software (www.r-project.org/), version 3.0.2, with pROC and timeROC R package for ROC analysis; car package for generalized linear models.

3. RESULTS

Below we reported results based on the age-based cut-off of 70 years. However, categorizing patients into EO and LO based on the 65 years cut-off provided very similar results (see Figures from e2 to e4 in the online supplementary materials).

3.1 Patients' descriptive features

We included 188 MCI patients with a mean follow-up of 28 (SD=14) months, of whom 76 were EO and 112 were LO. The ratio EO/LO is lower in ADNI than in EU datasets (22.5% vs 56.6%, $p<0.001$), indicating that European centres involved a younger population than the ADNI one. EO patients had higher MMSE scores both at baseline ($p=0.011$) and at the last follow-up ($p=0.009$) than LO patients, showing however a similar MMSE yearly change ($p=0.217$). Moreover, EO showed higher levels of CSF Aβ42 ($p=0.013$), while all the other biomarkers were similar between EO and LO patients ($p>0.05$) (Table 1). Similarly, we did not find any difference in the proportions of biomarker normality/abnormality at baseline between EO and LO patients (Table 1). More details about socio-demographic and clinical features by study centre are reported in Table e1 of the online supplementary materials.

3.2 Effect of age at onset on the biomarker ability to predict cognitive decline

Patients with abnormal biomarkers showed significantly different survival analysis distributions than patients with normal biomarkers. In EO patients, all the assessed biomarkers showed a significant ability to predict cognitive decline, namely patients with abnormal biomarkers at baseline developed cognitive decline more rapidly than those with normal biomarkers, with FDG-PET showing the best performance (FDG-PET: unadjusted HR=17.71, $p<0.001$; CSF Aβ42: 3.09, $p=0.010$; CSF t-tau: 2.57, $p=0.010$; Hippocampal Volume: 2.40, $p=0.015$; Figure 1A). In LO patients, all biomarkers but CSF t-tau were able to predict cognitive decline (FDG-PET: 3.19, $p<0.001$; CSF Aβ42: 2.41, $p=0.011$; Hippocampal Volume: 2.03, $p=0.009$; Figure 1B). For both EO and LO groups and all biomarkers but CSF Aβ42, HRs remained almost unchanged while adjusting for age, gender, APOE, baseline MMSE and study center. Similar results were obtained pooling toghether EO and LO MCI patients (whole MCI group: $p=0.001$ for FDG-PET; and $p<0.05$ for CSF t-tau, CSF Aβ42 and hippocampal volume).

When comparing prognostic performance to detect cognitive decline of each biomarker between EO and LO patients, FDG-PET showed a better ability to predict cognitive decline in EO than in LO patients (EO AUC=0.935 vs LO AUC=0.681, $p<0.001$), while the other biomarkers showed similar preformances in both groups ($p>0.05$) (Figure 2). When time-dependent ROC curves are applied, FDG-PET confirmed its high prognostic performance starting from time t=32 (Figure e1 in the online supplementary materials).

When combining all the biomarkers in a multiple model, in EO patients FDG-PET was the only factor significantly associated with cognitive decline ($p<0.001$), while in LO all biomarkers but CSF t-tau were associated

with cognitive decline (FDG-PET and hippocampal volume: $p<0.001$; CSF Aβ42: $p=0.021$). Moreover, the prognostic performance to detect cognitive decline of FDG-PET alone in EO patients was significantly higher than the one resulting from the combination of FDG-PET, hippocampal volume and CSF Aβ42 in LO patients (multiple models: EO AUC=0.935, LO AUC=0.753; $p<0.001$) (Figure 3).

3.3 Effect of age at onset on the biomarker ability to predict time-to-progression

FDG-PET and hippocampal volume were the only significant predictors of time-to-progression with $p=0.043$ and 0.032 respectively (Table 2). Moreover, the prognostic effect was statistically different between EO and LO only for FDG-PET (interaction term $p=0.040$), whereas this interaction showed only a trend to significance ($p=0.073$) for hippocampal volume. In particular, the negative beta coefficient of the factor Group means that in EO the time-to-progression was lower than in LO; moreover an increase of ten thousand of AD t-sum (index of Alzheimer-related hypometabolism) predicts 1.69 months earlier conversion in EO MCI, and 0.81 months later conversion in LO MCI (slope LO MCI=2.50-1.69=0.81): in others words, an enhancement of FDG-PET predicts a shorter time-to-progression in EO than in LO. Interestingly, when the linear models were adjusted for MMSE, gender and APOE, the prognostic effect on time-to-progression of FDG-PET and hippocampal volume were fully and partially mediate by APOE ($p=0.065$ and $p=0.037$ respectively). The advantage of these predictive models is that they allow to estimate, given socio-demographic and clinical features, the patient-specific time-to-progression.

4. DISCUSSION

In this study, we investigated the impact of age at symptom onset on the prognostic value of Alzheimer's biomarkers with respect to cognitive decline and its time-to-progression in a large sample of MCI patients homogeneous in terms of cognitive profile and impairment severity and all assessed by the four available biomarkers. These are two separate questions, considering that a biomarker predicting cognitive declines does not necessarily predict when cognition declines. We found that Alzheimer's biomarkers as taken alone are able to predict the developing of cognitive decline both in EO and LO groups (except for CSF t-tau in LO), with patients with abnormal biomarkers at baseline showing an increased incidence of cognitive decline over time than those with normal biomarkers. In addition, when combining all these biomarkers, FDG-PET is the only signifciant predictor of cognitive decline in EO patients, and its prognostic value in this group was greater than that one obtained combining all significant biomarkers in LO patients. Moreover, with regard to time to progression, we found that FDG-PET and hippocampal volume were associated with time-to-progression, but only FDG-PET showed a different prognostic value between EO and LO patients. These results are confirmed also in separated analyses for EU and ADNI cohorts where the ratio EO/LO is significantly different between them.

Our findings are in line with a paper showing that FDG-PET added greater prognostic information to routine clinical tests than CSF or MRI [10]. Previous studies compared biomarker diagnostic value in EO and LO patients with Alzheimer's disease, finding highest diagnostic value for MRI in LO [16], and for FDG-PET in EO patients with Alzheimer's disease [17]. There is only one previous study examining the effect of age on prediction of cognitive decline by MRI, CSF, and FDG-PET biomarkers in MCI patients [36]. Major findings were that in younger patients, CSF and MRI biomarkers correctly predicted about two thirds of the conversions, while FDG-PET was only a marginally significant predictor; in the older patients MRI predicted cognitive decline, but CSF biomarkers and FDG-PET did not.

As to time-to-progression, a previous study performed in MCI subjects with evidence of amyloid pathology found that CSF t-tau and hippocampal atrophy can predict time-to-progression [37]. To the best of our knowledge, this is the first study assessing the impact of age at onset on predicting time-to-progression by core biomarkers. Our results suggest that both hippocampal atrophy and FDG-PET can be used (alone and together with APOE information), to provide a magnitude of time-to-progression. Although APOE emerges as key role in the disease progression, the contribution of FDG-PET and hippocampal atrophy in predicting the time-to-conversion was still (at most) significant after APOE adjustment. This groundbreaking approach allows to estimate, based on patients features, age of onset and biomarker measured values, the remaining time free from disease and thus to allow neurologist/geriatrician to plan a specific and patient-oriented disease management. There are a number of findings deserving discussion. First, we

found FDG-PET to be the best predictor of cognitive decline in particular in EO. This is likely due to EO greater hypometabolism, suggesting that the disease could be more aggressive in EO than in LO patients, or even that EO and LO patients may have different underlying pathological processes, with the former group being more homogeneous with regard to the underlying AD pathology. Indeed, the higher prognostic value of FDG-PET in EO than in LO patients suggests that cortical hypometabolism measured with AD t-sum, a FDG-PET index of AD-related hypometabolism, has a higher accuracy in detecting the cognitive decline due to AD as compared to Aβ42, tau and hippocampal atrophy, which are common features also in neurodegenerative diseases other than AD [1]. Moreover, in EO, FDG-PET was the only factor significantly contributing to cognitive decline in the multiple model; while in LO all biomarkers but CSF t-tau significantly contributed. This may suggest that FDG-PET, hippocampal volume and CSF Aβ42 are closely related in EO, while in LO they all independently contribute. Last, amyloid marker did show prognostic value in both EO and LO. Despite amyloid being one of the first markers to become abnormal, amyloid negativity is associated with better outcome and amyloid positivity could be a risk factor at any age.

Current findings could have been influenced by the choice of biomarkers. Among established biomarkers of amyloid-β accumulation, we included CSF Aβ42 protein concentration while amyloid imaging biomarkers, despite being widely validated, were not included due to paucity of data available. However, amyloid PET and CSF Aβ42 are highly intercorrelated [38, 39], then we assume they would have led to similar results. Among biomarkers of neural degeneration or injury, we included CSF t-tau, FDG-PET and structural MRI biomarkers, while we decided not to include CSF phosphorylated tau due to the paucity of data available. As a biomarker denoting decreased FDG uptake on PET we chose a summary metric of Alzheimer-like hypometabolism [28], which was previously shown to provide good discrimination power between patients with Alzheimer's disease and controls [40, 41], and to be a valid marker to monitor the progression of MCI to dementia due to Alzheimer's disease [42]. Among structural MRI biomarkers, new diagnostic criteria suggest to consider atrophy in a specific topographic pattern involving medial, basal and lateral temporal lobe, and medial and lateral parietal cortices [2]. We included hippocampal volume, demonstrated to be a sensitive [43], and pathologically validated [44, 45] marker of neurodegeneration that parallels and precedes cognitive decline [46], and we computed it with an automated segmentation software (Freesurfer) to avoid bias due to different hippocampal segmentation protocols among centres.

This study has some strengths and limitations. The group of 188 MCI patients under study has all the 4 biomarkers available at baseline, paired with information on cognitive trajectories derived from long-term follow-up (28 months on average). Despite CSF biomarkers were a-posteriori normalised among centres, the fact of having biomarkers measured in different laboratories, probably using different strategies to derive cut-offs, could have affected the study results. Moreover, similar procedures for hippocampal atrophy assessment are not yet available, and

thresholds for abnormality currently vary from laboratory to laboratory, increasing error variance of the assignment to normality/abnormality. Moreover, the inclusion of data coming from different datasets (ADNI and EU) may be a source of heterogeneity. For this reason, we adjusted our Cox regression model by study centre and rerun the analyses on these two cohorts separately to further confirm our results. Cohorts analyses confirmed FDG-PET as the best biomarker in predicting the cognitive decline difference between normal and abnormal values for EO in both EU and ADNI cohorts. In LO patients, although the HR were comparable to the ones of whole sample, in some cases the results were quite far from significance and it was basically due to the sample size of the two separated cohorts EU and ADNI. Similar consideration holds for the time to progression analysis: beta coefficients are comparable (in sign and in value) to the ones of the whole sample even if their significance was affected by the sample size. These findings support the reliability of our results. Current findings could have been influenced by the choice of the age cut-off for the definition of elderly. Although the conventional threshold is 65 years, it is worth noting that the evidence on which this definition is based on is becoming even more feeble, especially in Western countries in which the 'rejuvenation' and slow-age phenomena have been seen [29–31]. The age distribution of our MCI sample is coherent with these phenomena. Moreover, a recent paper showing that the cut-off of 70 years differentiates between EO and LO groups in terms of neuropsychological, not biomarker profile, better than the cut-off of 65 years conventionally used [47]. In this sense , thus, our cut-off choice constitutes both a novelty and an incentive in considering new evidence-based definition of cut-off for discriminating between young and old population. In order to dispel all doubts regarding the effect of age cut-off on our study results, we repeated all analyses re-categorising patients based on the cut-off of 65 years, finding very similar results (see online supplementary materials). Moreover, we used age at diagnosis as proxy of age at onset, under the assumption that the moment of arrive to a memory clinic for cognitive impairment corresponds to the symptom onset. However, these moments do not always correspond. An undeniable limit of this work regards the definition of the study population based on a syndromic rather than a clinical diagnosis. Cognitive decline was defined based on multiple outcomes. The ideal outcome would have been the onset of dementia due to Alzheimer's disease at follow-up over an adequately long period; failing that, we included two additional outcomes (losing more than 3 points between first and last MMSE administration over a minimum time period or getting a score less than 24 at last MMSE administration), highly associated with having or being about to develop dementia. However, we cannot completely exclude that decline on the MMSE may also result from non-Alzheimer's dementia in the future after follow-up.

5. CONCLUSION

In conclusion, this study showed that all biomarkers are able to predict cognitive decline in both age groups (except for CSF t-tau in LO), but the prognostic value of FDG-PET in EO is greater than even all combined biomarkers in LO

patients. FDG-PET is also able to predict different patterns of time-to-progression between EO and LO patients, by providing, through predictive linear models, an estimate of time free from disease based on patient-specific biomarker levels and age of onset. These results indicate that FDG-PET may represent the most universal tool for the establishment of a prognosis in MCI patients. The current findings may contribute to the development of guidelines for the use of biomarkers in clinical settings.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflicts of interest

The authors have no conflicts of interest to report.

Ethical standards

This study was approved by the ethics committee of each participating center and all participants were enrolled after written informed consent was obtained.

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Table 1. Clinical and genetic features of 188 MCI patients categorized by age at onset (EO or LO).

	EO (≤ 70 years) N=76	**LO (> 70 years) N=112**	**EO vs LO *p value***
Clinical and genetic features			
Age at symptom onset (years)	64.6 (5.8)	78.4 (4.3)	<0.001
Gender (Females %)	41 (53.9%)	42 (37.5%)	0.038
Education (years)*	16.8 (2.9)	15.9 (2.8)	0.142
Follow-up time (months)	29.9 (17.6)	26.6 (11.4)	0.536
Baseline MMSE	27.3 (1.7)	26.7 (1.76)	0.011
Last follow-up MMSE§	25.7 (4.0)	24.3 (4.0)	0.009
MMSE yearly change§	-1.7 (4.6)	-2.0 (4.0)	0.217
ApoE ε4 carriers #	46 (62.2%)	53 (49.1%)	0.112
Biomarkers			
CSF Aβ42 (z)	0.01 (1.07)	-0.31 (1.07)	0.013
% Normal/ % abnormal	41/59	28/72	0.086
CSF total tau (z)	-0.40 (1.32)	-0.36 (1.34)	0.755
% Normal/ % abnormal	55/45	53/47	0.842
FDG-PET (AD t-sum)	21433.9 (23452.8)	16455.0 (16774.4)	0.754
% Normal/ % abnormal	55/45	64/36	0.276
Hippocampal volume (w)	-2.0 (2.4)	-2.1 (2.3)	0.989
% Normal/ % abnormal	59/41	61/39	0.956

Values are mean (standard deviations) or frequency (percentage). MMSE yearly change was computed with the formula (MMSE at the follow-up-MMSE at the baseline)/(follow-up time/12). Normal/abnormal classification as defined in Section suppl 1.2.

* missing data: 45 for EO, 23 for LO.

§ missing data for 4 EO and 11 LO.

missing data for 2 for EO, 4 for LO.

p denotes *p*-values of Mann-Whitney test (for continuous variables) or chi-squared test (for categorical variables).

Table 2. Prediction of time to conversion in 76 early onset (EO)[#] and 112 late onset (LO) MCI patients with cognitive decline in 2 years on average. Parameters were computed in linear regression models with time to conversion (in months) as dependent variable and biomarkers and group (EO vs LO) as independent variables. In the adjusted regression model, MMSE score, gender and APOE as added as furrher independent variables. When the interaction between biomarker and group was not significant, (p>0.1) the interaction term was removed from the model; similarly when in the adjusted models the independent variables are not significant. Ten thousand t-sum increase was associated with 1.69 months earlier conversion in EO MCI, and 0.81 months later conversion in LO MCI (slope LO MCI=2.50-1.69=0.81).

		Unadjusted models		***Adjusted Models***	
		Coefficients (95% CI)	***P value***	***Coefficients (95% CI)***	***P value****
CSF Aβ 42	z-score	-0.67 (-3.77 ; 2.43)	0.672	-1.75 (-4.96; 1.50)	0.286
	Group	-1.18 (-6.48 ; 4.11)	0.663	-0.68 (-6.04; 4.69)	0.805
CSF total tau	z-score	-1.09 (-2.72; 0.54)	0.193	-1.65 (-3.35; 0.04)	0.060
	Group	-1.86 (-7.13; 3.41)	0.491	-1.99 (-7.27; 3.30)	0.463
FDG-PET	t-sum/10,000	-1.69 (-3.29; -0.09)	0.043	-1.58 (-3.23; 0.07)	0.065
	Group	-8.18 (-17.48; 1.12)	0.090	-5.85 (-15.53; 3.83)	0.240
	t-sum/10,000 x Group	2.50 (0.17; 4.83)	0.040	1.90 (-0.52; 4.33)	0.128
Hippocampal Volume	w score	1.66 (0.18; 3.14)	0.032	1.68 (0.13; 3.24)	0.037
	Group	-6.74 (-14.54; 1.07)	0.094	-5.78 (-13.84; 2.29)	0.164
	w-score x Group	-2.09 (-4.34; 0.16)	0.073	-1.73 (-4.07; 0.62)	0.154

1 outlier in EO, identified by Tukey's Inter-Quantile Range of model residuals, was excluded from each biomarker model computation.
* In all adjusted models, APOE was the only significant factor with a pvalue ranged from 0.011 and 0.037 in the 4 models.

FIGURE LEGENDS

Figure 1. Survival curves displaying the association of biomarker abnormality with cognitive decline by age at onset in MCI patients (A: EO, B: LO). Patients with normal and abnormal biomarkers are denoted by dashed and solid lines, respectively. + indicates censored cases. HR: unadjusted hazard ratio (95% confidence interval) in Cox regression models. Adjusted HRs statistically differ from unadjusted HRs only for Aβ42 (in EO: adjHR=2.43 (0.81÷ 7.25), p=0.111; in LO adjHR=1.94 (0.98÷ 4.26), p=0.064). The most prognostic individual biomarker is FDG-PET with higher prognostic value in EO (HR=17.71) than in LO patients (HR=3.19).

Figure 2. Receiver operating characteristic curves showing prognostic performance (discrimination of cognitive decline) of biomarkers. Values denote areas under the curve (95% confidence interval). The analysis confirms the findings of Figure 1.

Figure 3. Receiver operating characteristic curves showing the prognostic performance for cognitive decline of risk scores computed by multiple Cox regression models including biomarkers with significant effect (earlier onset: FDG-PET only; later onset: Aβ42, FDG-PET, and hippocampal volume). Biomarkers were included in the model as continuous variables. Values denote areas under the curve (95% confidence interval). The two curves were significantly different (p<0.001).