Biomarkers and functional decline in prodromal Alzheimer's disease

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ABSTRACT

BACKGROUND: Little is known of possible associations between Alzheimer's disease (AD) biomarkers and instrumental activities of daily living (IADL) change over time.

OBJECTIVE: The present study seeks to identify relationships between baseline imaging and fluid biomarker profiles, and decline in IADL utilising data collated from the AD Neuroimaging Initiative (ADNI) cohort.

METHODS: Generalised estimating equations analysis, adjusted for cognitive deterioration, was applied to a cohort of 509 individuals from all stages of ADNI, including 156 healthy controls, 189 early mild cognitive impairment (MCI) patients and 164 MCI patients.

RESULTS: A significant correlation was found between baseline biomarkers - specifically CSF Aβ and FDG PET, and IADL change over a 3-year period in individuals with MCI. Importantly, comparable correlations between presence of pathological biomarker levels and temporal decline in both functional and cognitive performance were also noted.

DISCUSSION: We show that distinct baseline biomarkers may predict latent changes in IADL. Our results necessitate a revision of the commonly held view upholding cognitive changes as the predominant endpoint measure associated with presence of abnormal baseline biomarkers.

Keywords: Alzheimer's disease, biomarker, prediction, early diagnosis, activities of daily living, cerebrospinal fluid, positron emission tomography, magnetic resonance imaging

1. Introduction

The clinical stages of Alzheimer's disease (AD) are characterised by a slowly progressive memory-dominant cognitive deterioration, which is accompanied by a progressive impairment of normal instrumental activities of daily living (IADL) [1, 2] including complex activities such as managing finances, driving or using public transportation, undertaking household chores or doing the shopping [3]. Presence of early cognitive, and IADL changes are evident up to 10 years before an individual meets the diagnostic criteria for AD dementia [4], where evidence of significant cognitive and functional deterioration is required to establish AD diagnosis [5].

Until recently the *in vivo* diagnosis of AD was mainly based on clinical judgement, however newly proposed guidelines emphasise the use of biomarkers such as cerebrospinal fluid (CSF) and neuroimaging measures to identify the presence of AD pathology at different disease stages, as well as predict the progression from the earliest symptomatic stage, i.e. early mild cognitive impairment (eMCI) [6], to AD dementia [7, 8]. Although future cognitive decline can be predicted with relatively high accuracy using certain neuropsychological tests, established CSF, imaging and genetic biomarkers: total-Tau (t-tau), pospho-Tau_{181} (p-tau) and amyloid-$\beta_{1\text{-}42}$ (Aβ1-42), magnetic resonance imaging (MRI) mediotemporal lobe atrophy, 18F-fluorodeoxyglucose (FDG) positron-emission-tomography (PET) glucose metabolism, C11-Pittsburgh-Compound-B (PIB) PET fibrillar amyloid load and apolipoprotein-ε4 allele (*APOE*-ε4) [9-11], little is known about the association between presence of AD risk-biomarkers and IADL change over time.

It is well established that functional decline is commonly used as a co-primary endpoint in many clinical studies, along with cognitive decline [7, 8]. In addition, biomarkers are commonly used as surrogate endpoints in clinical drug trials, and have provided strong evidence of predicting cognitive decline over time. Recent Food and Drug Administration (FDA) guidelines [12] for AD clinical trials designed to capture participants at earlier disease stages, have asked that a combined cognitive and functional co-primary outcome measure approach be applied to demonstrate

efficacy, and ensure clinical meaningfulness of a cognitive benefit observed through drug treatment. Even though functional decline is considered an equally important and highly clinically relevant co-outcome measure, there is still very little evidence to show that baseline biomarker measures could serve as predictors of function decline (independent of cognitive decline). In order to reliably utilise AD biomarkers as surrogate endpoints in clinical trials involving functional and cognitive co-endpoint measures, it is critical to ascertain whether baseline biomarkers can indeed reliably predict functional decline independently of cognitive decline.

Previously published data suggest that early biomarker measures may indicate future IADL regression usually in late prodromal and early stage disease populations [13-16]. However to date, no study has sought to put together a comprehensive picture of how multiple baseline AD biomarker measures may impact on downstream IADL decline, specifically within a prodromal population. Furthermore, it is not clear if baseline biomarkers predict IADL change independently of baseline cognitive status, which itself is a strong factor influencing functional performance. This study utilises data obtained from the AD Neuroimaging Initiative (ADNI) cohort. By analysing data collected from patients in the earliest clinical stages of AD as well as healthy elderly controls; we examined the relationship between baseline imaging and fluid biomarker measures and IADL change over time through comparisons of functional deterioration in individuals with normal versus abnormal baseline biomarker measures.

2. Materials and methods

Data used in the preparation of this article were obtained from the ADNI database (adni.loni.usc.edu) on 25th March 2014. The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, PET, plus additional biological markers, clinical and neuropsychological assessment can be combined to measure the progression of MCI and early

clinical AD. ADNI was reviewed and approved by all host study site review boards, and participants completed informed consent after receiving a comprehensive description of ADNI. Participants were aged between 55-90 (inclusive), considered cognitively normal (CN), eMCI, MCI or AD dementia diagnosed individuals, and underwent serial evaluations of functional, biomedical, neuropsychological and clinical status at various intervals.

2.1 Study population

The current study utilised data collected at baseline and 24- to 36-months in 12-month increments. In cases where there were an N too small to consider at 36-months, follow-ups were only included to 24-months (review Supplementary Figure 1 & 2). Included were baseline data from CN subjects (defined as MMSE score between 25 and 30, inclusive; CDR score of 0; no evidence of depression; and no memory complaints; n=156), subjects with eMCI (n=189), and patients with MCI (n=164) from all stages of ADNI (ADNI 1 n=199, ADNI GO n=6, ADNI 2 n=377). Both eMCI and MCI were defined as MMSE score between 24 and 30, inclusive; CDR score of 0.5; report of memory complaints; no significant functional impairment. Additionally, to distinguish between the diagnostic groups: eMCI and MCI; objective memory deficits on the Wechsler Memory-Scale-Logical Memory II test was used with scores between 0.5SD-1.5SD depicting eMCI and lower than 1.5SD below the norm indicative of MCI. This ensured no overlap between the diagnostic groups. Included subjects had available structural MRI scans, FDG PET, CSF proteins (Aβ1-42, t-tau and p-tau) and *APOE*-ε4 allele carrier status (dichotomised into carriers vs non-carriers). Of the samples obtained, 113 participants were missing baseline MRI scans, hence, the 3-month follow up visit data was alternatively included. Additionally included were available free recall trial measures from the Rey Auditory Verbal Learning Test (RAVLT) [17] and a measure of IADL, the Functional Activities Questionnaire (FAQ) [18], both being obtained from each of the above mentioned visit times, up to 36-months, where available (Table 1). The RAVLT

measures verbal memory performance, where the sum of the five RAVLT free recall trials score was used; scores ranging from 0–75; lower scores on the RAVLT indicate greater memory impairment. This tool was chosen for its known sensitivity in measuring early signs of episodic memory decline; a key indicator of MCI due to AD [19-22]. The FAQ is an informant-based measure of IADL. Ratings range from normal (0) to dependent (3) on 10 subscales for a total of 30 points, with higher scores indicating more impaired functional status. An informant based questionnaire of functional performance far exceeds the cogency of a self-reporting version, with FAQ exhibiting highest validity in predicting AD related functional decline in multiple studies [2]. Further rationale for the utilisation of this instrument is reflected in a pioneering study reported by Teng and others, where the authors indicated that FAQ scores >6 were consistent with functional impairment [23].

2.2 Biomarkers

An in-depth description of biomarker acquisition and performance measures in ADNI can be obtained at www.loni.ucla.edu/ADNI, with image and CSF collection protocols available elsewhere [24-27]. Briefly, TaqMan quantitative polymerase chain reaction assays were used for genotyping *APOE* nucleotides 334 TC and 472 CT with an ABI 7900 real-time thermocycler (Applied Biosystems, Foster City, CA) using DNA freshly prepared from whole blood samples [28]. Mean FDG count was obtained per subject based on a composite region of interest in an AD typical hypometabolic pattern [24, 26]. FreeSurfer software (http://surfer.nmr.mgh.harvard.edu) was utilised to extract MRI (1.5 T) measured hippocampal volume where an atlas-based approach was implemented and has been validated for use in subjects with a great deal of morphologic variability. Uncorrected hippocampal volume for head size was used as a previous study showed that the association between hippocampal volumes and cognition was not altered by intracranial volume normalization [29]. Peptide CSF measures were generated from aliquot samples collected

at the same time [30] using commercially available enzyme-linked immunosorbent assays (ELISAs). Validated cut-offs were applied to a differential between normal and pathological findings for CSF p-tau and t-tau [26, 31-32], CSF Aβ [24, 31-33], FDG PET [24-26] and MRI hippocampal volume [24-26, 31-33].

2.3 Statistical analysis

Linear generalised estimating equations (GEE) were conducted to examine the influence of baseline biomarker measures on IADL deterioration. The same analysis was utilised to compare and contrast results with cognitive deterioration. In this report, time is represented such that repeated measurements are dependent samples, whereas measurement between patients are considered to be independent. Accordingly, FAQ and RAVLT were modeled as dependent variables, and baseline biomarker measures as independent variables in separate GEE analyses. Baseline biomarker measures where dichotomized into positive (abnormal) and negative (normal) measures, hence our analyses are based on these values and not the original biomarker measurement values. GEE uses all available data from all points in time (including baseline measures) to estimate a population-averaged effect of biomarkers on FAQ/RAVLT over time. GEE does not model the (relative) change of FAQ/RAVLT as a dependent variable but as original values obtained from each individual over time. Concerning the dependent variable, GEE accounts for dependency of observations measured in the same individual over time by using a working correlation structure. Selection of GEE as an appropriate statistical model for this study allowed an estimation of the temporal effect of baseline imaging and CSF AD-biomarker measures presence on IADL across the investigated population (population-averaged effects), as opposed to within subject effects. Additionally, the model took into account missing values across the time course. Additional information on GEE models may be viewed elsewhere [34, 35].

Longitudinal analyses were adjusted for age, education and *APOE* ε4-allele status. In addition, FAQ was adjusted for RAVLT at baseline and vice-versa. We did not adjust for gender due to identified studies reporting zero gender bias on variations in IADL performance over time [36]. Statistical two-sided significance level was set at 5% ($p<0.05$). To our knowledge, this study is the first to explore associations between baseline biomarkers and IADL change independently of cognitive decline, therefore our analyses are purely exploratory. Hence, we considered it appropriate not to adjust for multiple comparisons as this is only necessary for confirmatory analyses [37]. All analyses were performed using the gee and ggplot2-packages in R version 3.2.1 [38-40].

3. Results

Relevant sample characteristics are presented in Table 1.

A significant association was found between baseline FAQ and RAVLT scores ($p<0.001$), where the Spearman's correlation coefficient between baseline FAQ and RAVLT given as r=-0.40, however, FAQ and RAVLT scores after baseline were no longer comparable. Significant associations between IADL, cognitive performance and specific biomarkers were found in both eMCI and MCI groups prior to adjustment, but not in the CN group.

There were no significant associations among the eMCI patient group once results were adjusted for age, *APOE-ε4* carriage, education and respectively FAQ (GEE models for AVLT) or AVLT (GEE models for FAQ) (Table 2).

In MCI patients, worsening FAQ scores were found to be associated with impaired glucose metabolism [FDG-PET data] ($r=3.59$, $p<0.001$) and low levels of CSF Aβ ($r=2.97$, $p=0.028$). In addition, RAVLT decline among MCI patients significantly correlated with abnormal glucose metabolism at baseline ($r=-4.60$, $p=0.009$), low CSF Aβ ($r=-6.59$, $p<0.005$) and high levels of CSF p-tau ($r=-6.85$, $p<0.001$) (Table 2).

In healthy controls, no significant associations were found between any of the biomarkers and FAQ as well as RAVLT scores.

The associations between baseline biomarkers and FAQ scores for all investigated subjects are presented in Supplementary Figure 1, and for RAVLT in Supplementary Figure 2. The results indicate that FAQ decline is independent of RAVLT decline, as can be seen by the inconsistent coefficients, that is, the signs of the coefficients (RAVLT vs. FAQ) for the same biomarker are neither consensual (same direction; e.g. both positive) nor opposing. Moreover, relative differences between the two scales do not correlate after 12, 24 and 36 months (Spearman's correlation coefficient: <0.2), whereby a high correlation (<.05) between the relative differences (e.g. baseline vs 12-months) of the two scales would suggest a dependence between the FAQ and RAVLT scales.

4. Discussion

Identification of alternate clinical study endpoint measures for AD clinical trials remains a crucial endeavour to be pursued, in light of recent FDA guidelines advocating the implementation of a multi-outcome measure approach (utilising not only cognitive, but also functional outcome measures) for a more robust design when executing clinical trials investigating AD drug targets [12]. Although emerging evidence posits that functional decline may serve as an adjunctive primary outcome measure with cognitive deterioration, and results on baseline biomarkers reliably predicting latent cognitive decline are none too few; the data on associations between distinct baseline AD risk biomarkers and temporal changes in IADL remain very limited. In a bid to determine whether baseline biomarkers co-predict changes not only in cognitive, but also functional performance, we sought to explore the association between baseline AD biomarker measures and functional decline independently of cognitive decline over time in healthy, eMCI and MCI elderly individuals. We therefore aimed to observe whether functional decline would

significantly differ between individuals who present with positive (abnormal) versus negative (normal) biomarker measures at baseline. By utilising a sub-set of participant data from the ADNI cohort, we implemented GEE to critically evaluate notable associations between objective assessments of cognitive (RAVLT) and functional (FAQ) change in relation to distinct AD risk biomarkers collected at subject enrolment.

Our data, adjusted for baseline cognitive performance and APOE ε4-allele carriage, revealed that functional decline in the MCI subject group was significantly correlated with abnormal glucose metabolism (assessed using FDG-PET) and CSF Aβ. It is important to highlight that reported associations between these biomarkers (present at baseline) and FAQ scores over 36-months remained significant even after adjusting for baseline RAVLT scores, suggesting that these baseline biomarkers may predict functional change independently of baseline cognitive status. No significant adjusted associations between baseline biomarkers and functional decline in the eMCI group were observed.

Baseline biomarker measures as a predictive factor of functional decline is a novel area of research, with very few studies having explored such relationships and of those comparable; our findings are congruent [14-16, 41-43]. Two separate studies found regional cortical thinning and decreased CSF Aβ1-42 to predict IADL decline across the AD spectrum [15, 41]. Likewise, correlations between cerebral atrophy and IADL decline in MCI individuals have been reported [14], with rate of decline found to increase three fold in *APOE*-ε4 positive individuals [42]. Although not identified in our study, decreased hippocampal volume in MCI individuals too has been linked to reduced functional performance, compared to MCI individuals who do not exhibit this biomarker deficit [43]. However, once again baseline cognition was not controlled for. While not a primary aim, our results also replicate those found in relation to baseline biomarkers and cognitive decline. For example, a recent study found the combined temporal, lateral parietal, and

posterior cingulate hypo metabolism seen on FDG-PET as well as CSF Aβ1-42 and p-tau to be associated with cognitive decline in MCI and mild AD dementia patient groups [16].

Our data is an extension of these recent findings through the exploration of a more comprehensive range of biomarkers from the earliest AD stage, namely, individuals presenting with eMCI, a currently experimental subject group which in itself is a strength and novelty. Interestingly, there were no statistically significant associations observed between any of the investigated baseline biomarkers and functional decline in the eMCI cohort. A possible explanation for this finding could be the heterogeneous nature of both MCI and possibly more so the eMCI subjects in terms of AD related disease and symptom progression. eMCI is an experimental concept whereby likelihood of progression to AD dementia has not been extensively explored. A recent exploratory study utilising the ADNI cohort sought to define latent classes based on similar growth patterns between cognitive and functional decline using Growth Mixture Modelling (GMM) or person-centred modelling approach to identify baseline risk characteristics associated with the specified trajectories [44]. In doing so, they were able to create a decision tree using clinical predictors to ascertain GMM determined trajectories. Three trajectory classes (C) were identified; C1 (steepest decline), C2 (intermediate) and C3 (shallow decline). Of notable differences between C1 and C3 were the mean age (C1 = 74.7, C3 = 72.9), *APOE* ε4 carriage (C1 = 69.1%, C3 = 38.3%) and amyloid status (C1 = 92%, C3 = 48.2%). Differences in these same risk variables between the eMCI and MCI cohorts explored in this study are congruent to C3 and C1 group characteristics respectively, potentially explaining the reduced likelihood of functional decline in the eMCI cohort. Indeed both cognitive and functional decline was steeper in the C1 group versus the C3 group. A longer follow-up period may bring about observed functional decline based on baseline AD-biomarker positivity in the eMCI cohort, if this data were available.

To our knowledge this is the first study to explore the association between baseline biomarker measures and functional decline independently of baseline cognitive performance and

APOE ε4 status. Our study suggests a significant predictive value for baseline biomarkers on functional decline in MCI individuals, particularly with measures such as glucose metabolism (FDG-PET) and CSF Aβ (see Supplementary Figure 1). According to the current hypothetical model of AD related biomarker trajectories [45], reduced cerebral glucose metabolism as shown in FDG PET and lower CSF Aβ concentrations are all biomarker changes known to manifest during MCI disease stage. Such predictors are associated with conversion and likely reflect disease severity, i.e., how close an individual is to a significant clinical transition [45]. Hence, it may be expected that the associations are stronger for baseline FDG-PET and CSF Aβ measures with functional decline due to these biomarkers being a manifestation of a disease stage involving conversion from MCI to AD, and hence a natural follow-on of functional decline would be expected within this 36-month follow-up period.

Study limitations must be acknowledged; it is important to mention that the standardisation of biomarker cut-offs is currently limited and results often vary among laboratories. Ultimately, it will be necessary to interpret biomarker data in the context of well-established normative values. Positive or abnormal values should fall within reliable and valid pathological ranges. However, current cut-offs have been used in numerous ADNI studies, and appear to show reasonable validity for the purposes of this paper [24-26, 32, 33, 46]. Additionally, the ADNI cohort is namely white, middle class, educated and without any major comorbidity, thus, it would be important to repeat such a study with a larger and more widely represented demographic. In addition, our analyses do not take into account biomarker changes over time as AD pathophysiology progresses, which impacts on the association of biomarkers and cognitive as well as functional abilities at any given point in time during the course of AD. Furthermore, we did not explore the effects of co-morbidities and non-AD (e.g. cerebrovascular) brain changes on the studied clinical parameters. As a result, the lack of histopathological verification of the clinical diagnoses is another limitation, but the ADNI cohort is on purpose enriched with probable pre-dementia AD cases, evidenced by

the first autopsy studies [47]. In regards to the exploratory nature of this study, multiplicity was not taken into consideration, hence it is prudent to recognise that caution must be taken when reviewing the significant outcomes of this analysis, noting the observed exploratory correlations may indicate true correlations, whereby confidence in such inferences are strengthened by congruent findings elsewhere as discussed in the manuscript [37]. Certainly there is value in running a confirmatory study, where outcomes would be sufficiently informative whether or not significance is reached. Finally, there is some missing data, especially in later visit time-points as study participants drop off. This is a classic pattern observed in prospective clinical research, and more studies surrounding participant retention across the AD spectrum is needed.

In light of next steps, exploring IADL sub-category scores by dichotomizing into complex vs simple functional tasks for added sensitivity may yield stronger correlations with baseline biomarker measures. This may also assist in validating complex IADL change as a further clinical characteristic observed in eMCI. Studies have indeed found increased sensitivity of IADL scoring in the MCI demographic, once scores are split into complex and simple categories [48, 49]. Furthermore, it may be more practical to utilise a combination of biomarkers, potentially with different weights, than individual markers to predict functional decline, and the validation of such a composite biomarker index is another important step. Finally, there is a growing interest in the literature relating to AD plasma biomarkers and functional decline whereby further exploration is warranted and may bring about production of invaluable confirmatory studies.

5. Conclusion

Patient behavioural changes and inability to perform daily functional tasks are directly related to caregiver burden, and as such provide a more meaningful construct to caregivers, practitioners, and payers [50]. Hence, it is critical that functional decline be more fully understood in relation to disease stage and correlations with well-known AD biomarkers. These results yield promise in

supporting postulations that baseline biomarker measures may prove a significant indicator of projected functional decline, hence strengthening efficacy in utilising functional decline as a co-primary endpoint in AD clinical research.

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Table 1. Description of the study sample

		Diagnostic group		
	Overall	**Normal**	**eMCI**	**MCI**
	(N = 509)	**(n = 156)**	**(n = 189)**	**(n = 164)**
Age, *mean ± SD*	73.2 ± 7.1	74.8 ± 5.5	71.2 ± 7.5	74.0 ± 7.4
Gender, *male* (%)	58.0	57.7	55.0	61.6
FAQ, *Median (IQR)*				
Baseline	0.0 (3.0)*	0.0 (0.0)	1.0 (3.0)	2.0 (6.0)*
Month 12	0.0 (4.0)*	0.0 (0.0)*	1.0 (4.0)*	4.0 (9.0)*
Month 24	1.0 (4.0)*	0.0 (0.0)*	1.0 (3.0)*	6.0 (12.0)*
Month 36	2.0 (8.0)*	0.0 (0.75)*	1.0 (4.0)*	8.5 (16.0)*
RAVLT, *Median (IQR)*				
Baseline	37.0 (16.0)	44.0 (15.0)	37.0 (15.0)	30.0 (12.0)
12	37.0 (16.25)*	43.0 (14.0)*	38.0 (14.0)*	29.0 (11.0)*
24	37.0 (18.0)*	44.5 (14.25)*	37.0 (16.5)*	28.0 (13.0)*
36	34.0 (16.0)*	39.0 (10.5)*	37.0 (13.75)*	24.0 (12.0)*
Biomarkers, AD-positive (abnormal) (%)				
APOE ε4	39.1	23.1	38.6	54.9
FDG-PET (count value ≤1.21)	32.4	19.9	24.3	53.7
MRI hippocampal volume (≤3260 mm3)	30.5	16.0	23.3	52.4
CSF Aβ1-42 (≤192 pg/ml)	47.0	32.7	36.0	73.2
CSF p-tau181 (>23 pg/ml)	46.6	33.3	38.1	68.9
CSF ttau (≥66 pg/ml)	30.4	21.6	24.6	45.4

*: based on available cases specified (FAQ and/or AVLT scores were not ascertained at missed visit time-points); IQR: Interquartile Range; eMCI: early Mild Cognitive Impairment; MCI: Mild Cognitive Impairment; FAQ: Functional activity questionnaire (higher scores indicate greater functional impairment); RAVLT: Rey auditory verbal learning test (lower scores indicate greater memory impairment); Positive (abnormal): baseline biomarkers which measured within the pathological range; Negative (normal): baseline biomarkers which measured within the normal range; APOE: Apolipoprotien E; CSF: cerebrospinal fluid; FDG PET: [18F] fluorodeoxyglucose positron emission tomography; p-tau181: tau phosphorylated at threonine 181; Aβ (+): participants with β- amyloid 1-42 levels in cerebrospinal fluid (CSF) lower ≤192 pg/ml.

Table 2. Associations between FAQ and RAVLT progression with baseline AD-risk biomarkers in normal, eMCI and MCI participants

AD-Positive Biomarkers	RAVLT Regression coefficient	*p*-value (sig.<0.05)	FAQ Regression coefficient	*p*-value (sig.<0.05)
FDG-PET (count value ≤1.21)				
Normal	-3.680	0.620	0.242	1.000
eMCI	-0.027	1.000	1.382	0.426
MCI	-4.605	0.009*	3.592	<0.001*
MRI hippocampal volume (≤3260 mm3)				
Normal	1.227	1.000	0.769	1.000
eMCI	-0.623	1.000	1.578	0.222
MCI	-1.584	1.000	2.221	0.161
CSF Aβ1-42 (≤192 pg/ml)				
Normal	0.200	1.000	0.117	1.000
eMCI	-3.777	0.063	1.056	0.636
MCI	-6.594	0.005*	2.972	0.028*
CSF p-tau181 (>23 pg/ml)				
Normal	0.343	1.000	0.333	1.000
eMCI	-0.835	1.000	0.419	1.000
MCI	-6.850	<0.001*	0.421	1.000
CSF ttau (≥66 pg/ml)				
Normal	-1.741	1.000	0.804	1.000
eMCI	-0.666	1.000	1.083	1.000
MCI	-3.279	0.236	-0.339	1.000

*: $p \leq 0.05$ (GEE models were adjusted for age, *APOE ε4*, education, and respectively FAQ (GEE models for RAVLT) or RAVLT (GEE models for FAQ) at baseline)

eMCI: early Mild Cognitive Impairment; MCI: Mild Cognitive Impairment; FAQ: Functional activity questionnaire; RAVLT: Rey auditory verbal learning test; Positive (abnormal): baseline biomarkers which measured within the pathological range; Negative (normal): baseline biomarkers

which measured within the normal range; APOE: Apolipoprotien E; CSF: cerebrospinal fluid; FDG PET: [18F] fluorodeoxyglucose positron emission tomography; p-tau181: tau phosphorylated at threonine 181; t-tau: total tau measurement; CSF Aβ (+): participants with β- amyloid 1-42 levels in cerebrospinal fluid (CSF) lower ≤192 pg/ml.

Supplementary Figure 2. Temporal representation of RAVLT composite measurements in normal, eMCI and MCI subjects according to baseline biomarker profiles (raw and unadjusted values).

eMCI: early Mild Cognitive Impairment; MCI: Mild Cognitive Impairment; FAQ: Functional activity questionnaire; abnormal: baseline biomarkers which measured within the pathological range; normal: baseline biomarkers which measured within the normal range; CSF: cerebrospinal fluid; FDG PET: [18F] fluorodeoxyglucose positron emission tomography; p-tau181: tau phosphorylated at threonine 181; t-tau: total tau measurement; CSF Aβ positive: participants with β-amyloid 1-42 levels in cerebrospinal fluid (CSF) lower ≤192 pg/ml.