Received: 15 October 2020 | Accepted: 6 November 2020

DOI: 10.1111/ene.14639

european journal of neurology
the official journal of the european academy of neurology

ORIGINAL ARTICLE

Biomarker-based stability in limbic-predominant amnestic mild cognitive impairment

Giacomo Tondo[1,2] | Giulia Carli[1,2] | Roberto Santangelo[3] | Maria Vittoria Mattoli[4] | Luca Presotto[5] | Massimo Filippi[1,3] | Giuseppe Magnani[3] | Sandro Iannaccone[6] | Chiara Cerami[7,8] | Daniela Perani[1,2,5] | for the Alzheimer's Disease Neuroimaging Initiative

[1]Vita-Salute San Raffaele University, Milan, Italy

[2]In Vivo Human Molecular and Structural Neuroimaging Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy

[3]Department of Neurology and INSPE, San Raffaele Scientific Institute, Milan, Italy

[4]Department of Neuroscience, Imaging and Clinical Science, G. d'Annunzio University of Chieti-Pescara, Chieti, Italy

[5]Nuclear Medicine Unit, San Raffaele Hospital, Milan, Italy

[6]Clinical Neuroscience Department, San Raffaele Turro Hospital, Milan, Italy

[7]Scuola Universitaria Superiore IUSS, Pavia, Italy

[8]Cognitive Computational Neuroscience Research Unit, IRCCS Mondino Foundation, Pavia, Italy

Correspondence
Daniela Perani, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Vita-Salute San Raffaele University, 20132, Milan, Italy.
Email: perani.daniela@hsr.it

Funding information
Ministero della Salute, Grant/Award Number: Ricerca Finalizzata Progetto Reti Nazionale AD NET-2011-02346784

Abstract

Background: The amnestic presentation of mild cognitive impairment (aMCI) represents the most common prodromal stage of Alzheimer's disease (AD) dementia. There is, however, some evidence of aMCI with typical amnestic syndrome but showing long-term clinical stability. The ability to predict stability or progression to dementia in the aMCI condition is important, particularly for the selection of candidates in clinical trials. We aimed to establish the role of *in vivo* biomarkers, as assessed by cerebrospinal fluid (CSF) measures and [^{18}F]fluorodeoxyglucose (FDG)-positron emission tomography (PET) imaging, in predicting prognosis in a large aMCI cohort.

Methods: We conducted a retrospective study, including 142 aMCI subjects who had a long follow-up (4–19 years), baseline CSF data and [^{18}F]FDG-PET scans individually assessed by validated voxel-based procedures, classifying subjects into either limbic-predominant or AD-like hypometabolism patterns.

Results: The two aMCI cohorts were clinically comparable at baseline. At follow-up, the aMCI group with a limbic-predominant [^{18}F]FDG-PET pattern showed clinical stability over a very long follow-up (8.20 ± 3.30 years), no decline in Mini-Mental State Examination score, and only 7% conversion to dementia. Conversely, the aMCI group with an AD-like [^{18}F]FDG-PET pattern had a high rate of dementia progression (86%) over a shorter follow-up (6.47 ± 2.07 years). Individual [^{18}F]FDG-PET hypometabolism patterns predicted stability or conversion with high accuracy (area under the curve = 0.89), sensitivity (0.90) and specificity (0.89). In the limbic-predominant aMCI cohort, CSF biomarkers showed large variability and no prognostic value.

Conclusions: In a large series of clinically comparable subjects with aMCI at baseline, the specific [^{18}F]FDG-PET limbic-predominant hypometabolism pattern was associated with clinical stability, making progression to AD very unlikely. The identification of a

Abbreviations: Aβ, amyloid-β; AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; aMCI, amnestic mild cognitive impairment; ANOVA, analysis of variance; CDR, Cognitive Dementia Rating scale; CI, confidence interval; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; FAQ, Functional Activities Questionnaire; FDG, fluorodeoxyglucose; HR, hazard ratio; HSR, San Raffaele Hospital; IADL, instrumental activities of daily living; LATE, limbic-predominant age-related TDP-43 encephalopathy; MMSE, Mini-Mental State Examination; MCI, mild cognitive impairment; PET, positron emission tomography; p-tau, phosphorylated-tau; SD, standard deviation; SPM, statistical parametric mapping; t-tau, total tau.

Data used in the preparation of this article were also obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wpcontent/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

Giacomo Tondo and Giulia Carli contributed equally to the work.

biomarker-based benign course in aMCI subjects has important implications for prognosis and in planning clinical trials.

KEYWORDS
[^{18}F]FDG-PET, cerebrospinal fluid, limbic-predominant, mild cognitive impairment, tauopathy

INTRODUCTION

Mild cognitive impairment (MCI) is an intermediate condition between cognitive changes of normal aging and dementia [1]. MCI subjects may progress to Alzheimer's disease (AD) or to other neurodegenerative dementias, or may remain stable or even revert to normal cognition [2]. The selection of candidates for clinical trials in AD should be very accurate, in order to identify subjects clinically mimicking AD dementia or MCI due to AD, but who will remain stable over time [3]. The biomarker-based estimation of risk of conversion to dementia and the identification of MCI subjects with a benign course have important implications for prognosis and in planning clinical trials [4]. The progression from MCI to AD has been related to several biomarker characteristics [5]. Low cerebrospinal fluid (CSF) levels of amyloid-β $(Aβ)_{42}$ are valid proxies for amyloidosis in AD [6], while high CSF levels of phosphorylated tau (p-tau) and total tau (t-tau), targeting cerebral fibrillar tau deposition and neurodegeneration, respectively, are unspecific [7]. The presence of neurodegeneration assessed by magnetic resonance imaging is also not specific for AD [8–10]. [^{18}F]fluorodeoxyglucose (FDG)-positron emission tomography (PET), a biomarker of neuronal dysfunction associated with neurodegenerative processes, is able to predict conversion to dementia conditions [11–13] or long-term stability in patients with MCI [3]. In MCI, a negative [^{18}F]FDG-PET brain scan or, conversely, brain hypometabolism in temporoparietal regions, provides high accuracy in the prediction of clinical stability or conversion to AD dementia, respectively [3,14]. The amnestic presentation of MCI (aMCI) represents the most common prodromal stage of AD [1], with an annual conversion rate of up to 30% [15]. Limited but important evidence shows that aMCI subjects with a predominant amnestic syndrome of hippocampal type, associated with imaging features of medial temporal lobe dysfunction, are characterized by clinical stability over time [16–19]. Recently, our research group described aMCI subjects with long-lasting clinical stability or slow progression of episodic memory deficits, with no or limited evidence of cortical amyloid load and an [^{18}F]FDG-PET pattern of medial temporal lobe dysfunction at the individual level [20]. These results suggested non-AD pathology as the main trigger of neurodegeneration, such as argyrophilic grain disease, primary age-related tauopathy or limbic-predominant age-related TDP-43 encephalopathy (LATE). LATE has indeed been proposed as the prominent aetiology in suspected non-AD pathology subjects and in subjects with evidence of neurodegeneration without concomitant tauopathy, especially in the presence of focal temporal lobe dysfunction [21].

In the context of the above, there is a need to better describe clinical trajectories and to define clinical outcomes in the aMCI population using baseline biomarkers, such as [^{18}F]FDG-PET brain hypometabolism patterns.

In the present study, we aimed to define the role of *in vivo* biomarkers of neurodegeneration and pathology, as assessed by [^{18}F]FDG-PET and CSF measures, in a large aMCI cohort. We assessed the accuracy of [^{18}F]FDG-PET, the influence of CSF biomarkers and AT(N) classification (amyloid [A], tau [T] and neurodeneration [N]) in estimating outcomes. *In vivo* biomarkers are crucial for personalized medicine, in planning clinical trials, and for the choice of therapeutic approaches, especially in the case of MCI, in order to avoid detrimental diagnostic and prognostic mistakes.

METHODS

Subjects

Subjects were retrospectively included from the Neurology Departments at San Raffaele Hospital (HSR), Milan, Italy (HSR-aMCI cohort), and from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu), screening the ADNI-1, ADNI-GO and ADNI-2 phases (ADNI-aMCI cohort). The ADNI is a US public–private partnership launched in 2003 and led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to collect data on MCI subjects and AD patients, as well as on healthy controls, evaluating the combined prognostic value of several AD biomarkers and of clinical and neuropsychological assessments. For up-to-date information, see www.adni-info.org.

Inclusion criteria were: (i) aMCI diagnosis according to Petersen criteria [22]; (ii) observational time for disease duration ≥4 years, as an appropriate timeframe to detect progression from the aMCI condition to dementia; (iii) CSF measures at baseline; (iv) [^{18}F]FDG-PET scan performed at baseline and analysed using the optimized statistical parametric mapping (SPM) procedures [23,24] showing one of the two specific brain hypometabolism patterns, namely, temporo medial hypometabolism (limbic-predominant pattern) [20] or temporoparietal, posterior cingulate and precuneus hypometabolism (AD-like pattern) [24].

In detail, the HSR-aMCI cohort was obtained by screening 280 aMCI subjects. We excluded 176 subjects due to lack of baseline [^{18}F]FDG-PET scan or CSF analysis, and/or short follow-up (<4 years). By evaluating the [^{18}F]FDG-PET scan of the remaining

104 subjects with aMCI, we included in the present study only those subjects showing the limbic-predominant pattern (*N* = 40) or the AD-like pattern (*N* = 20), excluding subjects with normal [^{18}F]FDG-PET scans or patterns attributable to other neurodegenerative diseases (i.e., frontotemporal dementia, dementia with Lewy bodies, cortico-basal degeneration, and others). Similarly, the ADNI-aMCI cohort was created by excluding from the initial sample of 818 subjects with aMCI those with missing baseline CSF and [^{18}F]FDG-PET scans and/or a lack of an adequate follow-up period (≥4 years), resulting in a cohort of 247 patients with aMCI. From this sample, after evaluating the specific [^{18}F]FDG-PET single-subject metabolism patterns, we selected only the subjects with limbic-predominant aMCI (*N* = 40) or the AD-like aMCI (*N* = 42), thus excluding the subjects with normal [^{18}F]FDG-PET scans or patterns specific to other neurodegenerative diseases (i.e., frontotemporal dementia, dementia with Lewy bodies, cortico-basal degeneration, and others) (Figure S1).

Overall, the sample selection strategy led to the inclusion of 142 subjects with aMCI, of whom 80 (mean follow-up 8.2 ± 3.30 years) comprised the group with an [^{18}F]FDG-PET limbic-predominant hypometabolism pattern, and 62 (mean follow-up 6.47 ± 2.07 years) comprised the group with an [^{18}F]FDG-PET AD-like hypometabolism pattern (Table 1).

The study was approved by the San Raffaele Hospital Ethics Committee and performed in compliance with the Declaration of Helsinki for the protection of human subjects. Written informed consent was obtained from all participants.

Clinical and cognitive evaluation

The Mini-Mental State Examination (MMSE) and Clinical Dementia Rating scale (CDR) global score were available at baseline and at the last available follow-up to evaluate global cognitive status and

TABLE 1 Demographic, clinical and cerebrospinal fluid features in the subjects with amnestic mild cognitive impairment

	Limbic-predominant aMCI at baseline (*n* = 80)	AD-like aMCI at baseline (*n* = 62)	*p*	Limbic-predominant aMCI at follow-up (*n* = 80)	AD-like aMCI at follow-up (*n* = 62)	*p*
Female/male ratio, *n*/*n*	32/48	35/27	–	–	–	–
Education, years	13.69 ± 4.56	13.95 ± 4.57	0.733	–	–	–
Age, years	74.28 ± 5.40	71.14 ± 6.39	0.002	78.78 ± 5.26	73.76 ± 6.87	0.000
Disease duration, years	4.05 ± 2.44	4.05 ± 2.51	0.557	8.20 ± 3.30	6.47 ± 2.70	0.000
MMSE adjusted score	25.73 ± 2.06	25.40 ± 1.81	0.481	25.05 ± 2.58	19.67 ± 5.71	0.000
CDR global score	0.50 ± 0.00	0.50 ± 0.00	-	0.56 ± 0.34	0.70 ± 0.25	0.000
IADL questionnaire score[a]	6.77 ± 1.31	7.01 ± 1.55	0.199	6.33 ± 1.13	5.45 ± 1.99	0.111
FAQ[b]	4.14 ± 5.98	4.14 ± 4.03	0.239	6.85 ± 8.83	9.98 ± 6.69	0.009
CSF Aβ42 pathological levels, *n* (%)	43 (54)	55 (89)	–	–	–	–
CSF t-tau pathological levels, *n* (%)	35 (44)	40 (65)	–	–	–	–
CSF p-tau pathological levels, *n* (%)	51 (64)	58 (94)	–	–	–	–
t-tau/Aβ42 pathological ratio ,*n* (%)	56 (70)	56 (90)	–	–	–	–
p-tau/Aβ42 pathological ratio, *n* (%)	63 (79)	62 (100)	–	–	–	–
APOE 23, %	10	2	–	–	–	–
APOE 33, %	44	38	–	–	–	–
APOE 34, %	34	48	–	–	–	–
APOE 44, %	12	12	–	–	–	–

Note: Data are mean ± SD, unless otherwise indicated.

Abbreviations: AD, Alzheimer's disease; aMCI, amnestic mild cognitive impairment; APOE, apolipoprotein E; CDR, Cognitive Dementia Rating scale; CSF, cerebrospinal fluid; FAQ, Functional Activities Questionnaire; IADL, instrumental activities of daily living; MMSE, Mini-Mental State Examination; n, number of subjects; p-tau, phosphorylated tau; SD, standard deviation; t-tau, total tau.

[a]Only San Raffaele Hospital cohort (limbic-like, *N* = 40 and AD-like, *N* = 20).

[b]Only Alzheimer's Disease Neuroimaging Initiative cohort (limbic-like, *N* = 40 and AD-like, *N* = 42).

progression. An index of progression was also calculated as the number of MMSE points lost per year (MMSE score at follow-up – MMSE score at baseline/years of follow-up) [25]. Functional abilities were evaluated with the Instrumental Activities of Daily Living (IADL) questionnaire for the HSR-aMCI cohort and with the Functional Assessment Questionnaire (FAQ) for the ADNI-aMCI cohort. Clinical and cognitive baseline-to-follow-up differences in the aMCI cohorts were examined using one-way analysis of variance (ANOVA) and the Kruskal–Wallis test (statistical threshold set at $p < 0.05$).

[^{18}F]FDG-PET imaging

In the HSR-aMCI cohort, [^{18}F]FDG-PET acquisition was performed at the Nuclear Medicine Unit of the San Raffaele Hospital (Milan, Italy), in accordance with the European Association of Nuclear Medicine guidelines [26], with a Discovery STE multi-ring PET-computed tomography system (GE Medical Systems, Milwaukee, WI, USA). In the ADNI-aMCI cohort, raw [^{18}F]FDG-PET images obtained at baseline were downloaded from the ADNI database. The acquisition procedure is described in the "ADNI PET technical procedures manual, version 9.5" (http://adni.loni.usc.edu/wpcontent/uploads/2010/09/PET-Tech_Procedures_Manual_v9.5.pdf). Raw [^{18}F]FDG-PET images were downloaded from ADNI and pre-processed to obtain a single NIFTI file containing the last 15 min of PET acquisition.

Image pre-processing was performed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software), implemented in MATLAB (MathWorks, Sherborn, MA, USA). We adopted an optimized SPM procedure implementing a standardized SPM [^{18}F]FDG dementia-specific template [23] for spatial normalization of [^{18}F]FDG-PET scans. This optimized method has been validated in both MCI and dementia patients at the single-subject level, showing high accuracy and reliability in estimating specific metabolic patterns in different conditions [11,12,24]. Images were smoothed with an 8-mm full width at half maximum Gaussian kernel. To remove inter-subject global variation in PET intensity, proportional scaling was used, following a previously validated procedure [23].

Cerebrospinal fluid assessment

In the HSR-aMCI cohort, measurements of CSF $A\beta_{42}$, t-tau and p-tau levels were obtained using commercially available enzyme-linked immunosorbent assay (ELISA) kits, according to the manufacturer's protocol. Normal values were set as follows: ≥ 500 ng/L for $A\beta_{42}$, ≤ 450 ng/L (if age was 51–70 years) or < 500 ng/L (if age was >71 years) for t-tau and ≤ 61 ng/L for p-tau, according to the ELISA kit guidelines and literature recommendations [27].

In the ADNI-aMCI cohort, CSF $A\beta_{42}$, t-tau and p-tau levels were measured using the multiplex xMAP[1] Luminex platform (Luminex Corp, Austin, TX, USA) with the INNO-BIA AlzBio3 kit (Innogenetics, Ghent, Belgium) as described previously [28,29]. We used "UPENNBIOMK_MASTER" data files, setting the normal values at ≥ 192 pg/ml for $A\beta_{42}$, ≤ 93 pg/ml for t-tau and ≤ 23 pg/ml for p-tau, using previously defined cut-off values [28].

AT(N) evaluation

The AT(N) classification system, evaluating the available biomarkers, classified the whole aMCI sample into subjects with an AT(N) non-AD profile (i.e., A − T − [N+] and A − T + [N+]), and subjects with an AT(N) AD profile (i.e., A + T − [N+] and A + T + [N+]).

We considered, in the whole aMCI cohort, $A\beta_{42}$ and p-tau CSF levels to define amyloid and tau pathology, respectively. Brain hypometabolism, as detected by [^{18}F]FDG-PET, was considered a marker of neuronal injury, thus was present in both the AD-like and the limbic-predominant groups [30].

One-way ANOVA and the Kruskal–Wallis test were used to evaluate differences between the group with an aMCI with AT(N) AD profile and the group with an AT(N) non-AD profile (statistical threshold set at $p < 0.05$).

Statistical analysis

Analysis of single-subject [^{18}F]FDG-PET statistical parametric mapping

Each [^{18}F]FDG-PET single-subject scan was tested for brain "hypometabolism" using a two-sample *t*-test comparison with a validated [^{18}F]FDG-PET database of healthy controls ($N = 112$) on a voxel-by-voxel basis, including age as a covariate [23]. The statistical threshold was set at $p = 0.05$, family-wise error-corrected, with voxels of cluster extent (Kep) ≥ 100 voxels. This method was validated in subjects whose data were acquired with different PET scanners, allowing us to obtain comparable results from different cohorts [31]. The evaluation of each single-subject brain metabolic pattern was made by three experts in [^{18}F]FDG-PET brain imaging, blinded to the clinical data. The experts had near-perfect agreement in the SPM hypometabolism map (t-map) classification ('Cohen's kappa' > 0.95), thus the independent classifications were merged into a single variable (i.e., SPM t-map classification), in which the classification obtained from the majority of raters was considered to be final.

Predictive value of [^{18}F]FDG-PET

Clinical progression was defined according to changes in the latest follow-up diagnosis available in the HSR and ADNI databases, including stability or conversion from aMCI to dementia.

We estimated the predictive value of [^{18}F]FDG-PET SPM hypometabolism patterns for conversion or stability in the aMCI cohort. Hazard ratios (HRs) for the variable of interest, namely, [^{18}F]

FDG-PET, were estimated via a Cox proportional hazards model with a univariate approach. The threshold was set at $p < 0.05$, with a lower limit of 95% HR confidence interval (CI) >1 for risk factors, and an upper limit <1 for protective factors.

The prognostic performance of the [^{18}F]FDG-PET single-subject SPM t-maps in the risk of progression or stability was also evaluated by using measures of sensitivity, specificity and accuracy, considering the follow-up clinical diagnosis as the diagnostic reference. Receiver-operating characteristic curve analysis was performed to find the optimal cut-off to discriminate between stable aMCI and aMCI showing progression to dementia.

Predictive value of cerebrospinal fluid

Each CSF measure was dichotomously classified as positive or negative for AD according to validated ADNI and HSR cut-off values [27,28,32]. Dichotomic CSF measures of $A\beta_{42}$, t-tau and p-tau and t-tau/$A\beta_{42}$ and p-tau/$A\beta_{42}$ ratios were used as independent variables in separate regression models to avoid multi-collinearity. We estimated the predictive power of CSF biomarkers by means of multiple logistic regression models, with diagnosis at follow-up (stable aMCI vs. progression to dementia) as the dependent variable, including age, sex, education and MMSE adjusted score at baseline as variables of nuisance.

Other predictive biomarkers

We evaluated whether global cognitive efficiency (MMSE) and demographic variables (age, sex, education) at baseline were able to predict cognitive changes, by means of linear regression analysis, using the index of progression as the dependent variable, representing progression of cognitive deterioration at follow-up. The significance threshold was set at $p \leq 0.05$. We performed all statistical analyses using SPSS version 23.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Analysis of [^{18}F]FDG-PET statistical parametric mapping

Figure 1 shows results from the [^{18}F]FDG-PET SPM single-subject analysis: the limbic-predominant hypometabolism pattern (Figure 1a), and the AD-like pattern (Figure 1b).

Cognitive and clinical features at baseline

Table 1 shows baseline and follow-up cognitive features in the aMCI groups.

At baseline, all aMCI subjects had normal MMSE and CDR global score values, with no impairment in functional abilities (IADL questionnaire and FAQ), and no significant cognitive/clinical differences were observed between the limbic-predominant and AD-like aMCI subjects (Figure 2).

Cognitive and clinical features at follow-up

At the last available follow-up, limbic-predominant aMCI subjects did not show clinical and global cognitive changes in comparison to baseline, as measured by MMSE, CDR global score, the IADL questionnaire and the FAQ. A total of 74 subjects (93%) remained clinically stable and only six subjects (7%) converted to a diagnosis of AD dementia. Conversely, AD-like aMCI subjects showed significantly worsened follow-up MMSE scores compared to the baseline assessment and significant impairment in CDR global score and functional abilities (Table 1). Fifty-three of these (86%) converted to AD dementia, while nine subjects (14%) had a stable clinical profile. The follow-up evaluation revealed significantly worse scores in the MMSE, CDR global score and FAQ in AD-like aMCI cohort than in limbic-predominant aMCI cohort (Figure 2, Table 1). Limbic-predominant aMCI subjects had a significantly longer disease duration in comparison to AD-like aMCI subjects. As underlined by the MMSE Index of Progression, limbic-predominant aMCI was associated with no global cognitive decline in contrast to AD-like aMCI (MMSE points per year −0.20 ± 0.70 and −1.50 ± 1.43, respectively; $p < 0.001$).

No clinical variable of interest included in the analysis, that is, age, sex, educational level, MMSE at baseline or disease duration predicted stability or conversion to dementia.

Predictive value of [^{18}F]FDG-PET

The predictive value of [^{18}F]FDG-PET was evaluated with regard to clinical conversion or stability at follow-up in the whole aMCI cohort. [^{18}F]FDG-PET indicated that the AD-like hypometabolism pattern was strongly associated with a greater risk of clinical progression to dementia (HR 11.81 95% CI 5.06–27.55; $p < 0.0001$ [Figure 4a]). Very few patients with AD-like aMCI did not progress to dementia (9/62). Furthermore, very few limbic-predominant aMCI patients progressed clinically (6/80), leading to a highly accurate prediction of clinical stability for the limbic-predominant hypometabolism pattern. The predictive performance of [^{18}F]FDG-PET with regard to progression, as tested by receiver-operating characteristic curve analysis, yielded an overall high accuracy of 0.90 (95% CI 0.84–0.95), with high sensitivity 0.90 and specificity 0.89, in converter versus non-converter classification (Figure 4b).

Cerebrospinal fluid findings

The number of patients with pathological measures of $A\beta_{42}$, t-tau, p-tau, t-tau/$A\beta_{42}$ and p-tau/$A\beta_{42}$ ratios in the whole aMCI sample are shown in Table 1. CSF levels of $A\beta_{42}$, t-tau and p-tau showed high variability in limbic-predominant aMCI. 54% had pathological Aβ42 levels, while

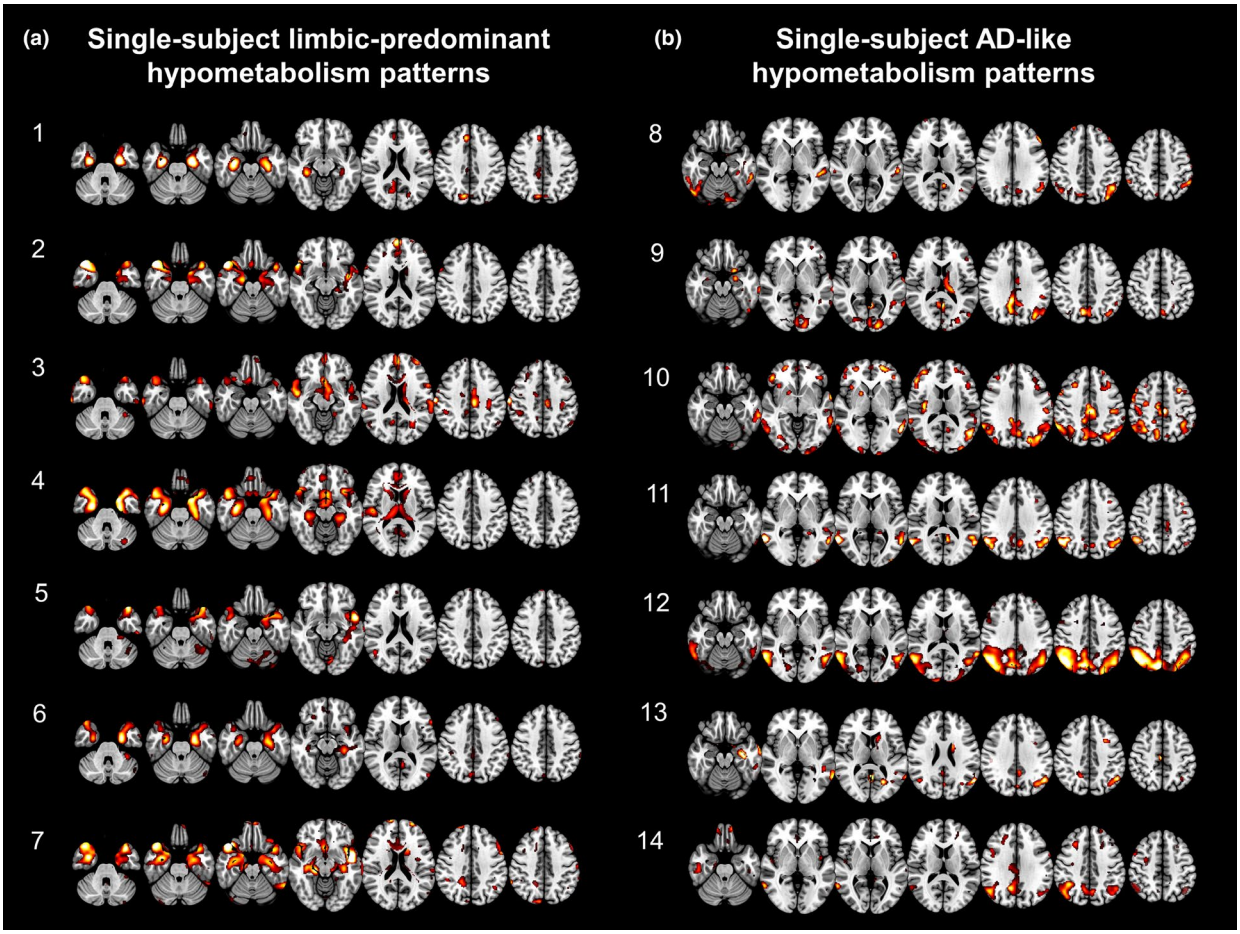

FIGURE 1 Examples of single-subject [^{18}F]fluorodeoxyglucose-positron emission tomography hypometabolic patterns. (a) Limbic-predominant amnestic mild cognitive impairment (aMCI): brain hypometabolism limited to the medial temporal structures in single cases (from 1 to 7), to a variable extent within other limbic structures. (b) Alzheimer's disease (AD)-like aMCI: temporoparietal AD-like hypometabolism in single cases (from 8 to 14). Statistical parametric mapping single-subject analysis: one patient versus 112 control subjects; $p < 0.05$, family-wise error-corrected at the voxel level with k > 100 voxels [Colour figure can be viewed at wileyonlinelibrary.com]

44% and 64% of subjects had pathological levels of t-tau and p-tau, respectively. Conversely, in 89% of the AD-like aMCI cohort, $A\beta_{42}$ CSF levels were low, whereas t-tau and p-tau were high in 65% and 94% of subjects, respectively. A pathological t-tau/$A\beta_{42}$ ratio was found in 70% of the limbic-predominant aMCI cohort and in 90% of the AD-like aMCI cohort, while a pathological p-tau/$A\beta_{42}$ ratio was present in 79% of the limbic-predominant aMCI cohort and in 100% of the AD-like aMCI cohort.

Cerebrospinal fluid predictive value

None of the CSF variables predicted stability or conversion to dementia in the limbic-predominant aMCI cohort. The AD-like aMCI cohort showed consistency in CSF biomarkers, indicating AD pathology.

AT(N) classification

According to the AT(N) classification, within the limbic-predominant aMCI cohort, 43 subjects (54%) were classified as having an AT(N) AD profile (32 subjects with A + T + [N+] and 11 subjects with A + T − [N+]), while 37 subjects (46%) had an AT(N) non-AD profile (19 A − T + [N+] and 18 A − T − [N+]; Figure 3). Of the AD-like aMCI cohort, 89% were classified as having an AD profile (51 subjects with A + T + [N+] and four subjects with A + T − [N+]), showing CSF evidence of amyloidopathy (Figure 3).

In the limbic-predominant aMCI cohort, no differences were found between the two groups with different likelihood of AD pathology (i.e., AT(N)-AD vs. non-AD profiles) in global cognitive functioning at baseline and follow-up evaluations, or in the index of progression.

DISCUSSION

The present results support differences in ‘he aMCI population, with one group having a stable clinical profile during a very long follow-up, [^{18}F]FDG-PET pattern of limbic-predominant hypometabolism and heterogeneous CSF biomarkers, and the other group showing a shorter disease duration with large conversion to AD dementia,

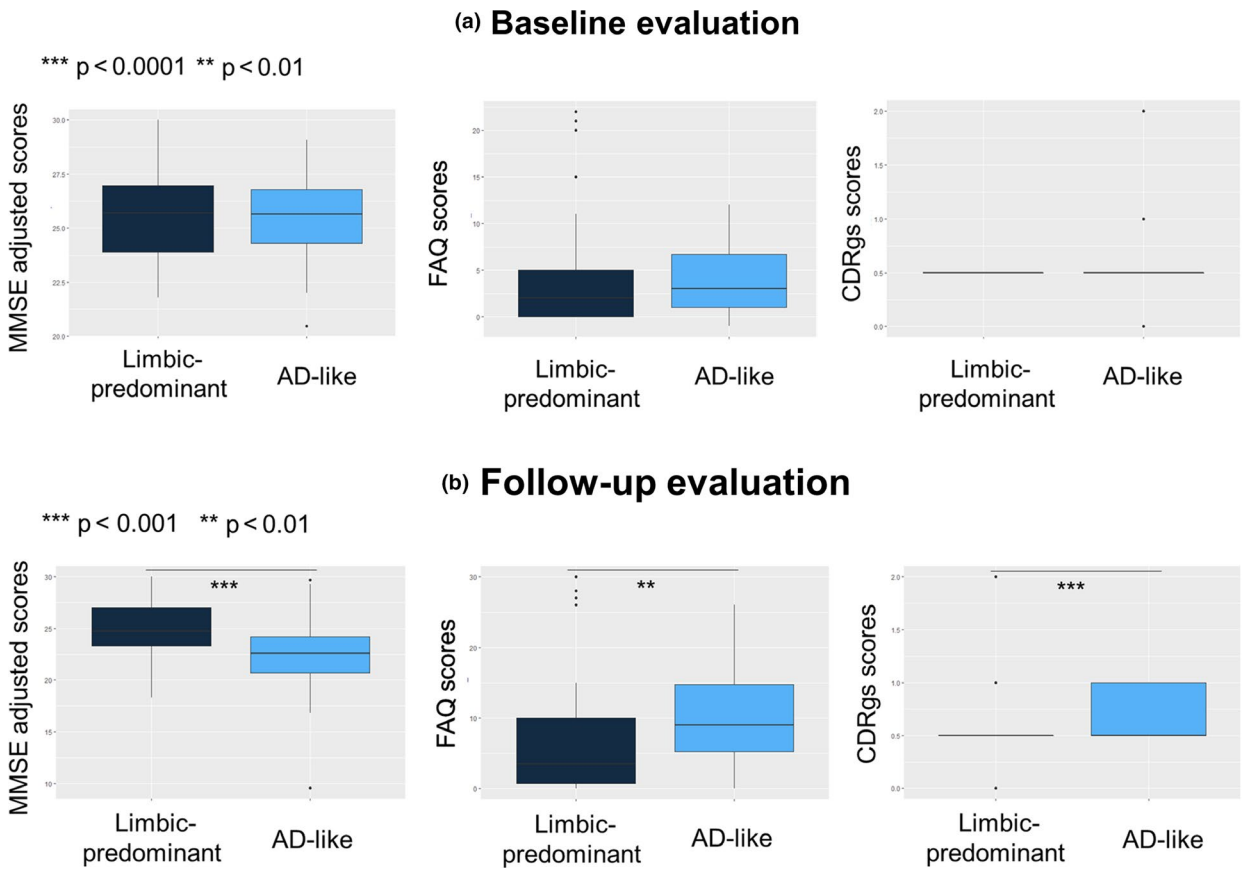

FIGURE 2 Cognitive and clinical baseline-to-follow-up differences between amnestic mild cognitive impairment (aMCI) cohorts. Global cognitive functioning differences between aMCI cohorts at (a) baseline and (b) follow-up evaluation, as measured by mean Mini-Mental State Examination (MMSE) and Functional Activities Questionnaire (FAQ) scores and Cognitive Dementia Rating scale (CDR) global scores. Follow-up scores were obtained from the last available follow-up evaluation in subjects who did not convert to dementia, and from the evaluation at time of conversion in subjects who converted to dementia. Limbic-predominant aMCI subjects did not show significant differences in global cognitive functioning as compared to those with Alzheimer's disease (AD)-like aMCI at baseline evaluation. Conversely, subjects with AD-like aMCI had a significantly lower MMSE adjusted score and higher FAQ score and CDR global score at follow-up evaluation [Colour figure can be viewed at wileyonlinelibrary.com]

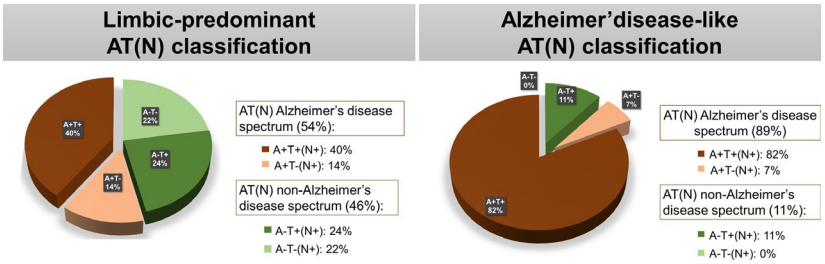

FIGURE 3 AT(N) classification (amyloid, tau, neurodegeneration) in the two amnestic mild cognitive impairment (aMCI) cohorts. Pie charts represent the percentage of limbic-predominant (a) and Alzheimer's disease (AD)-like (b) aMCI subjects with non-AD profile (dark green and light green) and AD profile (brick-red and light brick-red). The charts reflect the great variability in biomarker alterations in the limbic-predominant group, while an AD profile was prominent in the AD-like group [Colour figure can be viewed at wileyonlinelibrary.com]

typical AD-like hypometabolism pattern and CSF biomarkers suggesting AD pathology (Figure 1, Table 1).

At baseline, subjects in the two aMCI cohorts had the same amnestic phenotype, without differences in global cognitive functioning and functional abilities (Figure 2). The cognitive profile of the limbic-predominant aMCI group was comparable to that of previously reported cases affected by temporal lobe dysfunction, which were characterized by cognitive impairment strictly related to episodic memory, as well as a slower rate of cognitive decline than that observed in typical AD cases [17–19]. Clinical information alone is not accurate in anticipating the prognosis, stressing the need for a biomarker able to predict either disease progression or stability.

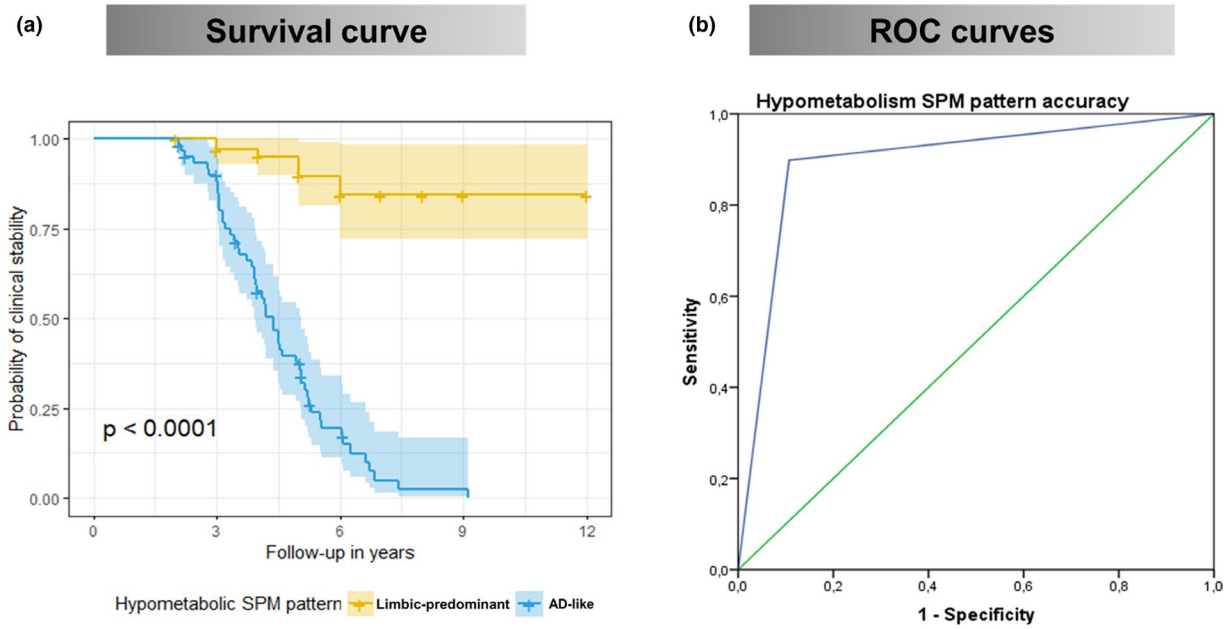

FIGURE 4 Survival and receiver-operating characteristic curves. (a) Survival curves indicating the probability of clinical stability during disease duration at time of conversion in subjects stratified according to [^{18}F]fluorodeoxyglucose (FDG)-positron emission tomography (PET) patterns, namely, the limbic-predominant (yellow) and Alzheimer's disease-like (blue) brain hypometabolism. (b) Accuracy of [^{18}F]FDG-PET single-subjects maps in predicting conversion toward dementia obtained with the receiver-operating characteristic curve analysis. SPM, statistical parametric mapping [Colour figure can be viewed at wileyonlinelibrary.com]

In this context, based on the typical temporoparietal pattern of hypometabolism in AD, [^{18}F]FDG-PET is recommended for evaluating subjects with MCI suspected of having underlying AD [13,33–36]. Notably, a negative [^{18}F]FDG-PET is associated with long-term clinical stability even in amyloid-positive individuals [3]. Our results indicate that [^{18}F]FDG-PET SPM classification was the most accurate biomarker, correctly differentiating subjects who converted to AD dementia from those who remained stable (Figure 4). Each subject with limbic-predominant aMCI shared the same non-AD brain hypometabolism pattern, with a focal vulnerability in the medial temporal lobes. The [^{18}F]FDG-PET limbic-predominant pattern, evaluated at single-subject level, was associated with an 80% chance of remaining clinically stable after up to 8 years of disease duration, strongly supporting a non-AD aetiology (Figure 4a). By contrast, the Cox proportional hazards model showed a significantly higher risk of conversion to dementia in those with aMCI with an AD-like hypometabolism pattern. Accordingly, [^{18}F]FDG-PET single-subject SPM presented high accuracy (0.90), sensitivity (0.90) and specificity (0.89) in classifying converters versus non-converters in aMCI populations (Figure 4b), in agreement with previous studies [3,13].

A crucial finding in the present study was the lack of correspondence between CSF biomarker alterations and clinical outcome. The CSF biomarkers, expressed both as single measures or ratios, were not able to predict prognosis in the limbic-predominant aMCI population. The great variability in CSF biomarkers (Table 1) suggests the presence of different possible aetiologies for neurodegeneration in our sample. Conversely, the aMCI subjects who converted to dementia presented a homogeneous CSF profile indicative of AD pathology. In the limbic-predominant aMCI cohort, the prognostic value of CSF and β-amyloidosis is null, while the specific [^{18}F]FDG-PET metabolic pattern confirms the reliability in predicting the absence of progression in these aMCI subjects, overcoming the role of amyloidopathy [3,20].

According to the AT(N) classification, aMCI subjects were grouped as having either an AD profile or non-AD profile [6]. Within the limbic-predominant aMCI group, 54% of subjects had a profile compatible with AD neuropathology changes, corresponding to the AD spectrum, whereas a considerable percentage (46%) of subjects were classified in the non-AD spectrum of disease (Figure 3) [6]. Notably, these two groups did not differ in the clinical follow-up, both showing stability.

In the limbic-predominant aMCI group, the clinical benign course over a long follow-up period and the [^{18}F]FDG-PET hypometabolic pattern exclude AD, suggesting different pathological substrates. These include neurodegenerative tauopathies, argyrophilic brain disease, hippocampal sclerosis and primary age-related tauopathy [37].

It has been suggested that, given the association of neurodegeneration with tauopathy in AD, in subjects whose neurodegenerative changes are due to non-AD comorbidity, LATE aetiology could be advocated [21]. LATE has been proposed as the prominent aetiology in subjects with suspected non-AD pathology and in subjects with evidence of neurodegeneration without concomitant tauopathy, especially in the presence of focal temporal lobe dysfunction [21]. Crucially, episodic memory deficits in LATE clinically mimic the level of impairment typical of AD. In addition, LATE and AD neuropathological changes can often coexist, increasing with older age.

In an autopsy series, Botha et al. [38] reported severe medial temporal lobe hypometabolism and pathological changes associated with LATE and hippocampal sclerosis in comparison with confirmed AD cases, which were associated instead with parietal and lateral/inferior temporal hypometabolism. Our results are in line with these findings, providing the additional diagnostic and prognostic value of [^{18}F]FDG-PET hypometabolism patterns in subjects with aMCI who showed stability/progression to dementia. Lastly, there was also some evidence of amyloidopathy in the limbic-predominant aMCI cohort, where the co-occurrence of mixed pathology or cerebrovascular pathology as the main factor responsible for this clinical picture cannot be excluded [39]. In a large autopsy series, a higher rate of mixed pathology was revealed in people with MCI showing a stable cognitive profile during their lifetime, while MCI converting to dementia was associated with a higher incidence of pure AD pathology [40].

A limitation of the present study was the lack of post mortem examination, which limits our ability to explain the aetiology conclusively. Nevertheless, this study implies major clinical findings. The limbic-predominant metabolism pattern is quite frequent in the aMCI population. By selecting subjects from large datasets, namely the HSR and the ADNI database, we revealed substantial frequency of this pattern, corresponding to 38% and 16% of the analysed cases, respectively (Figure S1). The previous literature showed that large proportions of patients with MCI may remain clinically stable, and population and community-based studies from different countries reported MCI stability incident rates ranging from 37% to 67% over the course of 1.5 to 5 years [41–44]. We can assume that a considerable proportion of stable MCI reported in previous studies also includes patients with limbic-predominant aMCI.

In conclusion, in aMCI, the specific neuronal dysfunction involving medial temporal lobes, as shown by [^{18}F]FDG-PET, can be considered a crucial biomarker, able to identify aMCI subjects who will not experience conversion to AD dementia even after long follow-up periods with high accuracy. No CSF biomarker was able to predict either stability or progression, revealing a poor diagnostic and prognostic role in this aMCI group. Our study indicates the high value of [^{18}F]FDG-PET in subject selection for clinical trials in AD and in the choice of therapeutic approaches, suggesting AD or non-AD classification, and providing biomarker features for stability/progression in aMCI subjects.

ACKNOWLEDGEMENTS

Data collection and sharing for this project was funded by the ADNI (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defence Alzheimer's Disease Neuroimaging Initiative award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, the Alzheimer's Association; the Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd. and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

CONFLICT OF INTEREST

The authors declare that they have no financial or other conflicts of interest.

AUTHOR CONTRIBUTIONS

Giacomo Tondo and Giulia Carli: analysed and interpreted the data, performed the statistical analyses, and wrote the manuscript. Luca Presotto: analysed data. Roberto Santangelo, Maria Vittoria Mattoli, Luca Presotto: acquired the data and revised the manuscript. Massimo Filippi, Giuseppe Magnani, Sandro Iannaccone and Chiara Cerami: acquired the data and revised the manuscript, adding important intellectual content. Daniela Perani: conceived the study, participated in its design and coordination, interpreted the data and revised the manuscript. All authors read and approved the final version of the manuscript.

FUNDING INFORMATION

This work was supported by the Italian Ministry of Health (Ricerca Finalizzata Progetto Reti Nazionale AD NET-2011-02346784).

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

All involved subjects gave written informed consent, in accordance with the declaration of Helsinki for protection of human subjects. For the ADNI cohort, the study was approved by local institutional ethics committees at each site (for up-to-date information, see www.adni-info.org). For the HSR cohort, the study was approved by the San Raffaele Hospital Ethics Committee. All patients from the HSR gave written informed consent to the study/data treatment for scientific purposes, including publications. The informed consents are available from the corresponding author on request.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

ORCID

Giacomo Tondo https://orcid.org/0000-0002-0057-3103
Roberto Santangelo https://orcid.org/0000-0002-8760-2703
Massimo Filippi https://orcid.org/0000-0002-5485-0479
Chiara Cerami https://orcid.org/0000-0003-1974-3421
Daniela Perani https://orcid.org/0000-0002-3421-204X

REFERENCES

1. Albert MS, DeKosky ST, Dickson D, 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. *Alzheimer's Dement*. 2011;7:270-279.
2. Petersen RC, Roberts RO, Knopman DS, et al. Mild cognitive impairment: ten years later. *Arch Neurol*. 2009;66:1447-1455.
3. Iaccarino L, Sala A, Perani D. Predicting long-term clinical stability in amyloid-positive subjects by FDG-PET. *Ann Clin Transl Neurol*. 2019;6:1113-1120. https://doi.org/10.1002/acn3.782
4. Sperling RA, Jack CR, Aisen PS. Testing the right target and right drug at the right stage. *Sci Transl Med*. 2011;3(111):111cm33.
5. Petersen RC, Lundt ES, Therneau TM, et al. Predicting progression to mild cognitive impairment. *Ann Neurol*. 2019;85:155-160.
6. Jack CR Jr, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimer's Dement*. 2018;14:535-562.
7. Bittar A, Bhatt N, Kayed R. Advances and considerations in AD tau-targeted immunotherapy. *Neurobiol Dis*. 2020;134:104707.
8. Barkhof F, Polvikoski TM, Van Straaten ECW, et al. The significance of medial temporal lobe atrophy: a postmortem MRI study in the very old. *Neurology*. 2007;69:1521-1527.
9. Raji CA, Lopez OL, Kuller LH, Carmichael OT, Becker JT. Age, Alzheimer disease, and brain structure. *Neurology*. 2009;73:1899-1905.
10. Jack CR, Dickson DW, Parisi JE, et al. Antemortem MRI findings correlate with hippocampal neuropathology in typical aging and dementia. *Neurology*. 2002;58:750-757.
11. Perani D, Cerami C, Caminiti SP, et al. Cross-validation of biomarkers for the early differential diagnosis and prognosis of dementia in a clinical setting. *J Nucl Med Mol Imaging*. 2016;43:499-508. https://doi.org/10.1007/s00259-015-3170-y
12. Cerami C, Della Rosa PA, Magnani G, et al. Brain metabolic maps in Mild Cognitive Impairment predict heterogeneity of progression to dementia. *NeuroImage Clin*. 2015;7:187-194.
13. Caminiti SP, Ballarini T, Sala A, et al. FDG-PET and CSF biomarker accuracy in prediction of conversion to different dementias in a large multicentre MCI cohort. *NeuroImage Clin*. 2018;18:167-177. https://doi.org/10.1016/j.nicl.2018.01.019
14. Frisoni GB, Boccardi M, Barkhof F, et al. Strategic roadmap for an early diagnosis of Alzheimer's disease based on biomarkers. *Lancet Neurol*. 2017;16:661-676.
15. Schmidtke K, Hermeneit S. High rate of conversion to Alzheimer's disease in a cohort of amnestic MCI patients. *Int Psychogeriatrics*. 2008;20:96-108.
16. Caffarra P, Venneri A. Isolated degenerative amnesia without dementia: an 8-year longitudinal study. *Neurocase*. 1996;2:99-106.
17. Lucchelli F, De Renzi E, Perani D, Fazio F. Primary amnesia of insidious onset with subsequent stabilisation. *J Neurol Neurosurg Psychiatry*. 1994;57:1366-1370.
18. Miceli G, Colosimo C, Daniele A, Marra C, Perani D, Fazio F. Isolated amnesia with slow onset and stable course, without ensuing dementia: MRI and PET data and a six-year neuropsychological follow-up. *Dement Geriatr Cogn Disord*. 1996;7:104-110.
19. Marra C, Villa G, Quaranta D, Valenza A, Vita MG, Gainotti G. Probable Alzheimer's disease patients presenting as "focal temporal lobe dysfunction" show a slow rate of cognitive decline. *J Int Neuropsychol Soc*. 2012;18:144-150.
20. Cerami C, Dodich A, Iannaccone S, et al. A biomarker study in long-lasting amnestic mild cognitive impairment. *Alzheimers Res Ther*. 2018;10:42.
21. Nelson PT, Dickson DW, Trojanowski JQ, et al. Limbic-predominant age-related TDP-43 encephalopathy (LATE): consensus working group report. *Brain*. 2019;142:1503-1527.
22. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med*. 2004;256:183-194. https://doi.org/10.1111/j.1365-2796.2004.01388.x
23. Della Rosa PA, Cerami C, Gallivanone F, et al. A standardized [18F]-FDG-PET template for spatial normalization in statistical parametric mapping of dementia. *Neuroinformatics*. 2014;12:575-593. https://doi.org/10.1007/s12021-014-9235-4
24. Perani D, Della Rosa PA, Cerami C, et al. Validation of an optimized SPM procedure for FDG-PET in dementia diagnosis in a clinical setting. *NeuroImage Clin*. 2014;6:445-454. https://doi.org/10.1016/j.nicl.2014.10.009
25. Caroli A, Prestia A, Galluzzi S, et al. Mild cognitive impairment with suspected nonamyloid pathology (SNAP): prediction of progression. *Neurology*. 2015;84:508-515.
26. Varrone A, Asenbaum S, Vander Borght T, et al. EANM procedure guidelines for PET brain imaging using [18F]FDG, version 2. *Eur J Nucl Med Mol Imaging*. 2009;36:2103-2110. https://doi.org/10.1007/s00259-009-1264-0
27. Sjögren M, Vanderstichele H, Ågren H, et al. Tau and Aβ42 in cerebrospinal fluid from healthy adults 21–93 years of age: establishment of reference values. *Clin Chem*. 2001;47:1776-1781.
28. Shaw LM, Vanderstichele H, Knapik-Czajka M, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol*. 2009;65:403-413.
29. Mattsson N, Insel PS, Donohue M, et al. Independent information from cerebrospinal fluid amyloid-β and florbetapir imaging in Alzheimer's disease. *Brain*. 2014;138:772-783.
30. Jack CR, Bennett DA, Blennow K, et al. A/T/N: an unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology*. 2016;87:539-547.
31. Presotto L, Ballarini T, Caminiti SP, Bettinardi V, Gianolli L, Perani D. Validation of 18 F-FDG-PET single-subject optimized SPM procedure with different PET scanners. *Neuroinformatics*. 2017;15:151-163.
32. Duits FH, Teunissen CE, Bouwman FH, et al. The cerebrospinal fluid "Alzheimer profile": easily said, but what does it mean? *Alzheimer's Dement*. 2014;10:713-723.
33. Laforce R Jr, Soucy J-P, Sellami L, et al. Molecular imaging in dementia: past, present, and future. *Alzheimer's Dement*. 2018;14:1522-1552.
34. Nobili F, Arbizu J, Bouwman F, et al. EANM-EAN recommendations for the use of brain 18 F-Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) in neurodegenerative cognitive impairment and dementia: Delphi consensus. *Eur J Neurol*. 2018;25:1201-1217.
35. Shaffer JL, Petrella JR, Sheldon FC, et al. Predicting cognitive decline in subjects at risk for Alzheimer disease by using combined cerebrospinal fluid, MR imaging, and PET biomarkers. *Radiology*. 2013;266:583-591.
36. Yuan Y, Gu Z-X, Wei W-S. Fluorodeoxyglucose–positron-emission tomography, single-photon emission tomography, and structural MR imaging for prediction of rapid conversion to Alzheimer disease in patients with mild cognitive impairment: a meta-analysis. *Am J Neuroradiol*. 2009;30:404-410.
37. Dani M, Brooks DJ, Edison P. Suspected non-Alzheimer's pathology–Is it non-Alzheimer's or non-amyloid? *Ageing Res Rev*. 2017;36:20-31.

38. Botha H, Mantyh WG, Murray ME, et al. FDG-PET in tau-negative amnestic dementia resembles that of autopsy-proven hippocampal sclerosis. *Brain*. 2018;141:1201-1217.
39. ten Kate M, Dicks E, Visser PJ, et al. Atrophy subtypes in prodromal Alzheimer's disease are associated with cognitive decline. *Brain*. 2018;141:3443-3456.
40. Abner EL, Kryscio RJ, Schmitt FA, et al. Outcomes after diagnosis of mild cognitive impairment in a large autopsy series. *Ann Neurol*. 2017;81:549-559.
41. Manly JJ, Tang M, Schupf N, Stern Y, Vonsattel JG, Mayeux R. Frequency and course of mild cognitive impairment in a multiethnic community. *Ann Neurol Off J Am Neurol Assoc Child Neurol Soc*. 2008;63:494-506.
42. Ravaglia G, Forti P, Montesi F, et al. Mild cognitive impairment: epidemiology and dementia risk in an elderly Italian population. *J Am Geriatr Soc*. 2008;56:51-58.
43. Roberts RO, Knopman DS, Mielke MM, et al. Higher risk of progression to dementia in mild cognitive impairment cases who revert to normal. *Neurology*. 2014;82:317-325.
44. Sachdev PS, Lipnicki DM, Crawford J, et al. Factors predicting reversion from mild cognitive impairment to normal cognitive functioning: a population-based study. *PLoS One*. 2013;8:e59649.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Tondo G, Carli G, Santangelo R, et al. Biomarker-based stability in limbic-predominant amnestic mild cognitive impairment. *Eur J Neurol*. 2021;28:1123–1133. https://doi.org/10.1111/ene.14639