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Biomarkers validation in Alzheimer's Disease and related pathologies

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Table of contents

Summary

The aim of this research project was to investigate biomarkers of Alzheimer's disease (AD) and related pathologies. The future of these biomarkers relies in their proper validation in all settings (analytical and clinical). In the first two papers, I cooperated with a task force of expert in AD and oncology field, who adapted a framework for biomarkers development used in oncology to AD. We assessed existing evidence based on this framework for amyloid-PET; CSF Abeta42, tau/phospho-tau; FDG-PET; hippocampal atrophy, ^{123}I-Ioflupane, ^{123}I-MIBG, and neuropsychology.

Then, I focused my PhD projects on one of the most reliable biomarkers of AD: brain metabolism by means of FDG PET and its clinical and validity among the phases of the roadmap framework. As FDG-PET relies its utility in AD not only as a diagnostic but also as a progression biomarker, we tried to use this powerful tool to clarify functional pattern able to predict future conversion to AD**.** One of the limitations of the latter studies was to have excluded from the sample patients with other disease than AD. This was done essentially for research purpose, but hampered the possibility to explore different metabolic patterns underpinning a mild cognitive impairment not due to AD. So, we explore the utility of FDGPET in the context of a relatively frequent syndrome who could mimic early symptoms of AD, DPD.

Our results were: by means of strategic five-phase roadmap, sufficient evidence of analytical validity (phase 1) is available for all biomarkers, but their clinical validity (phases 2 and 3) and clinical utility (phases 4 and 5) are incomplete.

In order to assess the accuracy of FDG PET in discriminating MCI patients who converted to AD from those who did not, we found that MCI patients not converting to AD within a minimum follow-up time of 5 years and MCI patients converting within 5 years,

baseline FDG PET and volume based analysis identified those who converted with an accuracy of 89%.

With the aim to identify the cortical regions where hypometabolism can predict the speed of conversion to dementia in MCIAD we found a diagnostic-pattern. This is a further, independent source of heterogeneity in MCI-AD and affects a primary-endpoint on interventional clinical trials (time of conversion to dementia).

Finally, with the aim to explore the role of FDG PET in the diagnosis of DPD, we compared brain FDG-PET among DPD patients, patients with early AD and normal subjects. We found that DPD patients had a specific relative hypometabolism both on caudate nuclei and right anterior cingulate (BA 25). This study confirms the role of FDG PET in the diagnosis of DPD and pave the way of a better understanding of its underpinning biological alterations.

In conclusion, during my PhD I explored the necessity to define the validity of biomarkers of AD and related pathologies in patients with MCI: from operative and analytic point of view, to clinical settings. Then I focused on the real clinical implication of one of the most useful and reliable biomarkers, FDG PET. Our findings support its role of a robust progression biomarker even in a naturalistic population, and underline its importance in the early diagnosis of AD.

Introduction

In 1906, Alois Alzheimer (1) defined the disease that was later to carry his name as an irreversible, progressive brain disorder which leads to dementia. This medical condition includes a progressive cognitive impairment and its neuropathological hallmarks are represented by senile plaques and neurofibrillary tangles, which could be identified postmortem in the grey matter of the brain. In the following years, plaques and neurofibrillary tangles were found to be composed of β-amyloid and hyperphosphorylated tau (p-tau) protein, respectively (2,3) and these processes lead to synaptic and neuronal loss, followed by clinical symptoms (4,5). The first criteria from the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease (AD) and Related Disorders Association for the diagnosis of AD were developed in 1984 (6), diagnosis at that time was based on clinical findings exclusively, with measurement of biomarkers, especially brain imaging biomarkers, recommended only to exclude other causes of cognitive decline. Over the past few years, the study of AD and other neurodegenerative diseases has significantly evolved, in terms of understanding of disease characteristics and evolution, also because of the increasing use of new biomarkers, and diagnostic criteria have clearly integrated this knowledge (7,8,9). The diagnosis with biomarkers, used alongside traditional neuropsychological tests, has led to a global improvement in accuracy and diagnostic earliness in memory clinics (10). But, in the current clinical practice, the use of biomarkers is often limited to selected cases for which the clinical diagnosis is problematic: atypical presentation, early age at onset, relevant comorbidities limiting the information that can be obtained by clinical and anamnestic evaluations (11). By contrast, the extensive use of biomarkers in the clinical diagnosis has been repeatedly proposed, in the assessment of cognitive complaints, because early and accurate diagnosis of even typical AD could be still challenging for clinicians (12) Furthermore, biomarkers are not useful only in the diagnostic

process, but they are associated also with disease progression. So they may capture biological responses to pharmacological or non-pharmacological interventions. AD biomarkers include neuroimaging tests and laboratory parameters aimed to detect direct and indirect signs of amyloid beta deposition and neurofibrillary neurodegeneration (13).

These extra- (amyloid) and intra- (tau) cellular lesions, which lead to synaptic dysfunction and neuronal death, may be identified also in the prodromal phase, which can last many years, that precedes the clinical onset of AD. Methods of detection include evidence of brain amyloidosis and tau deposits from amyloid (14) and tau ligands uptake at PET imaging (15,16), or indirect evidence such as the altered concentrations of the Abeta42 and tau proteins in CSF specimens (17). Also, functional (FDG-PET, e.g. temporo-parietal hypometabolism; 18) and structural neuroimaging (MRI) could identify synaptic dysfunction and loss of brain integrity. In particular, medial temporal atrophy, assessed either visually (19) or quantitatively (20) has great prognostic value. Finally, other assays tapping pathophysiological processes (namely, degeneration of the dopaminergic nigrostriatal pathway with 123IMIBG scintigraphy - 21, and myocardial postganglionic sympathetic dysfunction with 123I-Ioflupane SPECT - 22) may be used to exclude non-AD degenerative disorders (e.g., Lewy body dementia). The contribution of these AD biomarkers to improve the accuracy of the clinical diagnosis depends on the demonstration of their analytical validity, that is their ability to detect the key pathological hallmarks of AD and the correlated brain damage and dysfunctions. Extensive evidence of analytical and early clinical validity for these biomarkers led to them being integrated into research diagnostic criteria, with the aim of moving from an exclusionary approach in differential diagnosis to a positive diagnosis (7,8,9). Biomarkers can indeed enhance the accuracy of clinical diagnosis of AD, but we need to demonstrate their clinical validity before implementing their use in clinical settings. In fact, by now there is a wide evidence of analytical validity of biomarkers, but there is only a partial

evidence of their clinical validity and clinical utility (23), and this hampers the systematic use of biomarkers in ordinary clinical contexts. The available empirical evidence on the analytical and clinical validity of the afore mentioned AD biomarkers has been presented in six reviews reported in the same issue of Neurobiology of aging (24-29). The availability of in vivo measures of AD pathology of proven analytical validity has the transformative potential to provide a diagnosis of AD based on a clinico-biological rather than clinico-pathological basis. Moreover, because the neuropathology underlying AD accumulates gradually over several decades and the insidious onset of the disease reflects the long induction and latency periods (30), the possibility to accurately measure AD-related brain changes in vivo can substantially contribute to the detection of AD at the preclinical stage when future curative treatments might be more efficacious. The biomarker-based diagnosis is being used to inform better study designs, particularly for the selection and inclusion of participants for experimental studies, testing potential beneficial effects of interventions targeting specific disease mechanisms. This use of biomarkers will definitely increase the power of clinical trials; however, their accuracy will critically influence the appropriateness of subjects' selection and, thus, the final power of these studies. Changes in biomarkers can already be seen in the mild cognitive impairment stage, when functional disability is absent (31), and new diagnostic criteria now allow for a diagnosis of Alzheimer's disease to be made at the prodromal stage, before the development of full-blown dementia (7,8,32,33) Indeed, in order to maximize benefits from biomarkers clinical validation process, the population of interest should be non-proactively screened but composed by patients referred to a memory clinic or e specialist health service, by a general practitioner. These patients should have initial cognitive and behavioral deficiency, and they are referred as Mild Cognitive Impairment (MCI). This term describes a population with acquired cognitive impairment and no functional disability. The most typical MCI patient is one who has memory impairment beyond what is felt to be normal

for age but is relatively intact in other cognitive domains. More recently, the concept of MCI has been expanded to include other types of cognitive impairment beyond memory. In fact, mild cognitive impairment can be heterogeneous from both clinical and etiological perspectives. From the first perspective, heterogeneity may refer to the clinical presentation of MCI. Based on whether predominant memory impairment was present or not, two primary subtypes were delineated: amnestic and non-amnestic MCI. The amnestic form of MCI is most common, and most of the literature on the topic refers to this form of the disorder. Less common presentation could involve subjects being slightly impaired in multiple cognitive domains, called multiple-domain MCI. Both may show a slight impairment in activities of daily living as well but not of enough magnitude for the clinician to call the subject demented. In the multiple-domain form of MCI, subjects may have slight memory impairment in conjunction with mild impairments, for example, in executive function and language. A third clinical variety of MCI could involve a mild impairment in a single non-memory cognitive domain. This form of MCI, known as single non-memory-domain MCI is characterized by a person having a relatively isolated impairment in a single non memory domain such as executive function, visuospatial processing, or language. Depending of the MCI type, progression to dementia, of AD type or other forms of dementia

It is easy to comprehend why is important to define the identikit of MCI due to AD, or better, the criteria to define prodromal AD (34-36). First, clinical presentation should include a new-onset cognitive dysfunction reported by the patient, relatives, or physician that have lasted for at least the previous 6 months, particularly for episodic memory, and occasionally difficulty with language, visuospatial tasks, or topographic orientation. The independence should be maintained in completing daily activities, although some may be performed to a lower standard than previously (eg, not as efficiently or with help). The presence of major behavioral disturbances should be excluded, because, if so, it should be considered other

diagnoses such frontotemporal lobar degeneration or dementia with Lewy-bodies. By contrast, mild disturbances in the form of sleep disorders, apathy, or depression does not affect the consistency or diagnosis of Prodromal AD. The neurological examination results should be normal; if parkinsonism is present, differential diagnoses should be considered (e.g. dementia with Lewy-bodies, rare genetic forms of Alzheimer's disease, or frontotemporal lobar degeneration). The Mini-Mental State Examination score should be in the range of 24–30 and there should be consistent abnormal performance compared with mean age-specific and education-specific values on memory tests. Symptoms should be unexplained by psychiatric history and assessment.

Also, structural imaging and laboratory examinations should exclude non-degenerative and metabolic causes. From this view, medial temporal (mainly hippocampal) atrophy on MRI supports a neurodegenerative process that can suggest Alzheimer's disease, but also other disorders; on the other hand, atypical (neocortical) presentations might spare the medial temporal regions, especially in patients younger than 65 years. By contrast, reduced cortical metabolism on ^{18}F-fluorodeoxyglucose PET in posterior cingulate-precuneus and temporoparietal cortex increases the likelihood that Alzheimer's disease is the cause of cognitive impairment, whereas normal PET findings suggest no neurodegenerative disease. Also abnormal CSF protein concentrations indicating abnormal amyloid metabolism (low fibrillar β-amyloid [Aβ]42 concentration or a low ratio of Aβ42 to Aβ40) and neuronal damage (high total tau and hyperphosphorylated tau concentrations) increase the likelihood that Alzheimer's disease is the cause of the cognitive impairment, whereas the combination of a normal Aβ42 concentration and normal Aβ42:Aβ40 in CSF make Alzheimer's disease very unlikely. Furthermore, absence of brain amyloidosis on amyloid PET (using tracers such as florbetapir, flutemetamol, and florbetaben) makes Alzheimer's disease a very unlikely cause of cognitive impairment, whereas positive amyloid PET supports Alzheimer's disease

as the cause in young patients because the a priori risk of being amyloid positive is lower than in older people, among whom a substantial proportion of cognitively intact individuals are amyloid positive. Indeed, a positive result for one amyloidosis biomarker and one neurodegeneration biomarker is strongly associated with clinical progression over time and the development of disability and dementia within 5–7 years.

In fact, in recent years efforts have been directed examining conversion rates from MCI to AD. It has been reported that approximately 2% to 25% of those carrying the diagnosis of MCI converted to AD per year (37,38). While aMCI is the subgroup who have the greater risk to convert to AD, we must emphasize that same MCI patients can remain stable over time or even spontaneously improve their cognitive performance.

Over the other biomarkers, the one who have demonstrated more predictive ability for conversion is FDG PET, a highly relevant prognostic information for daily clinical use (39-41). FDG- PET is a functional neuroimaging technique which permit to measure in a quantitative (or semi-quantitative) way the local metabolism of the synaptic terminals in their neuron-astrocyte functional unit by labeling glucose with [18F]. In fact, it is known that the greater part of glucose utilization happens at the synaptic level. FDG-PET has been reported to be highly sensitive to metabolism reduction in AD patients over time, in follow-up studies lasting 1 year (42). This means that it is a suitable marker to follow the disease evolution and, consequently, to evaluate the potential effect of both symptomatic and neuroprotective agents (43). It has been computed that metabolism reduction in critical regions is in the order of 16–19% over 3 years, while in healthy subjects is virtually absent in such a time span (44). Furthermore, FDG PET plays its role in detecting by showing (or not) specific disease patterns, which are strongly connected with functional deficit. Indeed, FDG PET could be considered a polyhedral biomarker, either in the characterization of progression in AD, than in the differential diagnosis from other pathologies. Its current integration as a useful

diagnostic marker in clinical practice for selected situations already in the MCI phase (45). However, as for the other AD biomarkers its maturity for standard use in the clinical routine has never been systematically evaluated. Its role could be indeed pivotal in those entities that mimic early signs of AD, which diagnosis could be challenging by means of only clinical interview. When describing, as above, the identikit of typical prodromal AD patient, presence of a mild depressive trait is not a rare condition. Older adults with depression frequently complain about cognitive disturbances, and they are often diagnosed with MCI (46) But, following criteria for MCI diagnosis, these cognitive symptoms should be unexplained by psychiatric history and assessment (34-36). A misunderstood depressive trait could indeed mimic prodromal AD, a condition sometimes called Depressive Pseudodementia (DPD). This is a relative uncommon syndrome, but its accurate detection should be not considered as trivial because in contrast to cognitive deficits in neurodegenerative disease, depression-related cognitive impairment can be reversed (47). But, to the best of our knowledge, no former studies have been conducted on the role of FDG-PET in the diagnosis of DPD (48).

The aim of this research project was, first, to systematically check the evidence currently available on the use of biomarker for clinical and prodromal AD, by using a systematic framework. We attest the clinical and analytical validity of FDG PET as a core AD biomarker, defining its role first as a progression biomarker, then, as a reliable diagnostic biomarker capable to highlight the underpinning biological mechanism of AD and other related pathologies.

Study 1) Strategic roadmap for an early diagnosis of Alzheimer's disease based on biomarkers.

Background. In the past decade, the diagnosis of AD has made use of different biomarkers, which are now used by researchers in their diagnostic criteria (7, 31). Although these research criteria are not supposed to be used in clinical settings, many academic memory clinics use these biomarkers in routine practice to help assessment and management of patients. Without a consistent framework to assess the validity of biomarkers for Alzheimer's disease, however, their use has been heterogeneous and reimbursement by providers of health insurance inconsistent. Both factors are negatively affecting the provision of high-quality care to patients because the informative value of biomarkers cannot be used with full reliability in clinical practice.

In this policy view, we summarize the conclusions and recommendations from an interdisciplinary academic effort to set up a strategic research agenda, or roadmap, to accelerate the adoption of biomarkers for the diagnosis of Alzheimer's disease in clinical practice. The aim of the roadmap is to define a strategic research agenda to synchronize research efforts and complete validation effectively, to get biomarkers approval for clinical use.

Methods. An international task force (experts on AD and cancer biomarkers, scientific societies, patient advocates, and regulators) set out to identify the gaps of evidence to full clinical validity of AD biomarkers and define actions into a coherent and cost-effective roadmap. The development and use of biomarkers for screening and delivery of individualized treatment in oncological patients are much more advanced than those in people with Alzheimer's disease. In 2001, Pepe and colleagues (49) devised a five-phase framework for the development of biomarkers to screen for cancer in the general population. Each phase had one or two primary aims with pertinent outcome measures,

as well as several secondary aims. From this oncology framework, we have made adaptations to create a similar framework for Alzheimer's disease. Several basic differences in biomarker validation must be taken into consideration due to the more advanced knowledge available in oncology, and to reflect the current understanding of Alzheimer's disease (50). First, Alzheimer's disease biomarkers are intended for diagnosis rather than screening in the general population. Second, the access to brain samples at autopsy to obtain neuropathological data can be difficult, although neuropathology is the current gold standard for Alzheimer's disease diagnosis. Third, interventions that alter the course of Alzheimer's disease positively and, therefore, can have substantial effects on phase 5 outcomes (mortality, morbidity, and disability associated with Alzheimer's disease), do not yet exist. Within this background, we worked on these 5 sequential phases including: phase 1, preclinical exploratory studies; phase 2, clinical assay development for Alzheimer's disease pathology; phase 3, retrospective studies of longitudinal data available in repositories; phase 4, prospective diagnostic accuracy studies; phase 5, disease burden reduction studies (Table 1). Current maturity of biomarkers according to this framework was assessed from available literature on: Neuropsychology; MTA; 18F-FDG PET; CSF measures (Aβ42, tau, p-tau); 11C-PIB and 18F amyloid ligands PET. Tau PET was not included, because it is an emerging technology still in the earliest stages of development (51) However, the roadmap we propose provides a general framework that will be applicable to other technologies or techniques, including tau PET.

Results. We have reviewed evidence on the validity of Alzheimer's disease biomarkers, restricting the clinical context to the diagnosis of prodromal Alzheimer's disease. We summarize the conclusions of our literature review for each potential biomarker and highlight research priorities. The methods and results of this evidence review have been reported in detail (24-29,50).

Neuropsychology: Cerami and colleagues (24) focused on delayed free and cued recall tasks because these represent the most sensitive measures of memory decline in the typical presentation of Alzheimer's disease and have a reasonable degree of specificity for the typical dysfunction of Alzheimer's disease. They found that multiple tests are available to assess the same brain function, but they are not standardized for administration, scoring, and normative values. Research priorities, therefore, are to compare the diagnostic accuracy of these tests and harmonize them by consensus into a standard test with multilingual versions and pertinent normative values. The standardized test should include parts that would be sensitive for atypical presentations of Alzheimer's disease.

Medial temporal atrophy: Ten Kate and colleagues (29) reviewed the evidence for two different ways of assessing medial temporal atrophy: visual rating, and volumetric assessment. Medial temporal atrophy is the only biomarker for which phase 1 and phase 2 studies are almost completed. Only sparse preliminary data on the practical feasibility (phases 4 and 5) of the visual assessment of medial temporal atrophy are available. Moreover, like other Alzheimer's disease biomarkers, this assessment has limited specificity when used alone, and its usefulness in combination with other biomarkers must be assessed in phases 4 and 5. The major weakness of this biomarker is its poor specificity to distinguish non-Alzheimer's causes of cognitive impairment. Research priorities are to define standard operating procedures for automated algorithms and to rerun phase 3 studies to assess automated hippocampal volumetric analysis (table 1.3).

^{18}F-FDG PET: this biomarker is also at a more advanced stage of validation than most other Alzheimer's disease biomarkers (26). However, the greater availability of phase 4 preliminary data on the clinical relevance and cost-effectiveness of ^{18}F-FDG PET in patients with atypical or early-onset Alzheimer's disease is weakened by incomplete earlier phases.

CSF measures: CSF biomarkers for Alzheimer's disease are at an advanced stage of development (27) However, the available manual immunoassays are sufficiently stable only when used in experienced laboratories with quality control procedures. The fully automated assays (such as electrochemiluminescence), is a potentially important advancement, but standardized optimum protocol for preanalytical handling of CSF samples needs to be developed and implemented. The definition of cutoff values for normal ranges will need to be defined for all immunoassays with use of a suitable reference (preferably neuropathology).

Amyloid PET: Despite consensus on the equivalence of the three regulatory-approved fluorinated tracers used for amyloid PET, the interpretation of findings from studies in phase 3 is complicated by imperfect standardization of comparative reading or quantification procedures (28) Reliability of results might improve when a harmonized procedure for all tracers is more widely implemented. These uncertainties notwithstanding, findings from some small-scale phase 4 studies are already available, and collaborative efforts between researchers and tracer developers have led to ongoing larger-scale phase 4 studies (IDEAS and AMYPAD).

Discussion and conclusions. Phase 1 is complete for all biomarkers, and research priorities have been identified for phases 2 and 3, including the definition of standard procedures for reliable assessment, investigation of confounders affecting biomarker performance and thresholds, and comparison with other biomarkers to define an effective diagnostic algorithm. Proposed future action are to complete phases 2 and 3 according to proposed research priorities, set up phase 4 then phase 5 studies, and define guidelines for best use of biomarkers and of combinations thereof in clinical practice. Recommendations are to set up validation of new biomarkers according to the five phase framework and rerun validation studies for available biomarkers lacking evidence from phase 2; biomarkers should be validated in phase 4 studies done in qualified memory

clinics and after obtaining appropriate ethics approval and patients' informed consent.

Paper: Strategic roadmap for an early diagnosis of Alzheimer's disease based on biomarkers. Frisoni GB, Boccardi M, Barkhof F, Blennow K, Cappa S, Chiotis K, Démonet JF, Garibotto V, Giannakopoulos P, Gietl A, Hansson O, Herholz K, Jack CR Jr, Nobili F, Nordberg A, Snyder HM, Ten Kate M, Varrone A, Albanese E, Becker S, Bossuyt P, Carrillo MC, Cerami C, Dubois B, Gallo V, Giacobini E, Gold G, Hurst S, Lönneborg A, Lovblad KO, Mattsson N, Molinuevo JL, Monsch AU, Mosimann U, Padovani A, **Picco A,** Porteri C, Ratib O, Saint-Aubert L, Scerri C, Scheltens P, Schott JM, Sonni I, Teipel S, Vineis P, Visser PJ, Yasui Y, Winblad B. Lancet Neurol. 2017 Aug;16(8):661-676.

	Primary and secondary aims	Adaptations from oncology to AD
Phase 1: preclinical exploratory studies	Primary aims: (1) identify leads for potentially useful biomarkers; (2) prioritise identified leads	No substantial change
Phase 2: clinical assay development for Alzheimer's disease pathology	Primary aims: (1) estimate the frequency of true-positive and false-positive results or ROC, and assess ability to distinguish individuals with and without Alzheimer's dementia Secondary aims: (1) optimise procedures for assays and their reproducibility within and between laboratories; (2) determine the relation between phase 1 biomarker measurements made in tissues and those made in phase 2 studies in non-invasively collected clinical specimens; (3) assess variables (eg, sex and age) associated with biomarker status or concentration in controls (eg, healthy individuals);* (4) assess variables, especially disease characteristics, associated with biomarker status or level	Established disease in cancer is believed to correspond to overt dementia in AD; the preferable standard of reference in AD is pathology, although AD dementia is acceptable if there is reason to believe that most individuals being assessed have AD pathology (eg, NINCDS-ADRDA probable AD dementia)[3]
Phase 3: retrospective studies using longitudinal data available in repositories	Primary aims: (1) assess the capacity of the biomarker to detect early disease†; (2) define criteria for a positive screening test in preparation for phase 4 Secondary aims: (1) explore the effects of covariates on the discriminatory abilities of the biomarker before clinical diagnosis; (2) compare biomarkers to select the most promising; (3) develop algorithms for likelihood of positive results based on combinations of biomarkers; (4) determine required interval between biomarker testing if repeated testing is of interest in phase 4	In contrast to phase 3 studies in oncology, which are retrospective, nested, case-control studies, AD requires prospective longitudinal repository studies, in which the biomarker is measured at baseline in individuals with MCI and AD status ascertained at follow-up, preferably by AD pathology, but also by incident AD dementia or cognitive progression; as in cancer, AD biomarker results would not be used for diagnosis or treatment
Phase 4: prospective diagnostic accuracy studies	Primary aims: (1) determine the accuracy of core biomarkers in the clinical setting by calculating frequencies of positive and false-positive detection Secondary aims: (1) describe the characteristics of disease detected by the biomarker test, particularly with regard to potential benefits incurred by early detection; (2) assess the feasibility of implementing case-finding programmes and likely adherence of individuals with positive test results to work-up schedules and treatment recommendations; (3) make preliminary assessments of the effects of biomarker testing on disease-associated costs and mortality; (4) monitor disease diagnosed clinically but not detected by biomarker testing	The major difference with phase 4 in oncology is that studies will not involve clinically asymptomatic individuals; AD studies would include symptomatic but non-demented (MCI) patients and, therefore, would need to be done in highly specialised clinics that have guidelines for the collection, measurement, and interpretation of biomarkers; as in oncology, AD biomarker results would be used for diagnosis and treatment
Phase 5: disease burden reduction studies	Primary aims: (1) estimate reductions in mortality, morbidity, and disability associated with biomarker testing Secondary aims: (1) obtain information about costs of biomarker testing and treatment and per life saved or quality-adjusted life year gained; (2) assess adherence to testing and work-up in various settings; (3) compare different biomarker testing protocols, approaches to treating test-positive individuals in regards to effects on mortality, costs, or both	No adaptation needed, although the achievement of phase 5 outcomes is unlikely until treatments able to delay progression are available

Table 1.1 Five-phase framework to develop biomarkers for early diagnosis of Alzheimer's disease.

Key: AD=Alzheimer's disease. ROC=receiver operating curve. NINCDS-ADRDA=National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association. MCI=mild cognitive impairment. *Thresholds might need to be defined separately for different target subpopulations. †MCI or prodromal AD.

	Phase 1: preclinical exploratory studies; PA	Phase 2: clinical assay development for Alzheimer's disease pathology					Phase 3: retrospective studies using longitudinal data available in repositories						Phase 4: prospective diagnostic accuracy studies					Phase 5: disease burden reduction studies; PA
		PA	SA1	SA2	SA3	SA4	PA1	PA2	SA1	SA2	SA3	SA4	PA	SA1	SA2	SA3	SA4	
MRI medial temporal atrophy*	Full	Full	Part	Full	Full	Full	Full	PE	Part	Part	Part	NA	NE	NE	NE	NE	NE	NE
^{18}F-fluorodeoxy-glucose PET	Full	Full	Full	Full	Full	Part	Full	Part	PE	Part	Part	PE	NE	PE	NE	PE	NE	NE
^{11}C-PiB and fluorinated tracers for amyloid PET†	Full	Full	Part	Full	Part	Part	Full	Part	NE	Part	Part	PE	NE	NE	NE	NE	NE	NE
CSF measures (Aβ42 or Aβ42:Aβ40 or total tau and hyperphosphorylated tau)	Full	Full	PE	Full	Part	Part	Full	Part	Part	Part	Part	PE	PE	NE	NE	NE	NE	NE

Table 1.2: State of completion of biomarkers development in Alzheimer's disease for the five phases in the strategic roadmap

Key: PA=primary aim. SA=secondary aim. Full=Phase fully achieved (no need to collect further evidence). Part=Phase partly achieved (studies available but replication or completion is required). PE=only preliminary evidence available. NA=not applicable. NE=no evidence available. PiB=Pittsburgh compound. Aβ=fibrillar β-amyloid. *Assessments represent the least developed level between visual and volumetric medial temporal atrophy. †Using tracers such as florbetapir, flutemetamol, or florbetaben.

	Phase 2			Phase 3					
	SA1	SA3	SA4	PA1	PA2	SA1	SA2	SA3	SA4
Neuropsychology	Define standard neuropsychology tests, sensitive also to atypical AD presentations	Establish normative values	NP	NP	Define threshold to proceed with biomarker testing	NP	NP	NP	NP
Hippocampal volume	Define SOPs for automated algorithms	NP	NP	Assess prognostic accuracy	Threshold definition	Assess impact of covariates on diagnostic accuracy	NP	NP	NP
^{18}F-fluorodeoxy-glucose PET	NP	NP	Assess effects of covariates on retention	Reassess diagnostic accuracy	Harmonise and validate reading criteria; define threshold	NP	NP	NP	NP
CSF Aβ42 or Aβ42:Aβ40 or total tau and hyper-phosphorylated tau	Standardise preanalytical handling; validate fully automated immunoassays	Assess effects of non-AD pathologies on concentrations	Complete assessment of effects of covariates on concentrations	NP	Reassess thresholds with newly validated standards	NP	Redefine optimum combination of biomarkers for newly validated standards	Define optimum combination with other biomarkers	Determine within-individual changes over time with newly validated standards
Amyloid PET*	Assess comparability, reproducibility SOPs, and readout methods	Assess effects of covariates on retention	Assess effects of disease characteristics and covariates on retention	Reassess diagnostic accuracy if new standard is defined	Harmonise reading criteria and improve threshold definition	Assess impact of covariates on diagnostic accuracy	Compare with other biomarkers (mainly in CSF) with newly validated standards	Define optimum combination with other biomarkers	Determine meaning of intermediate or dubious retention and set interval between repeated testing if useful

Table 1.3: Research priorities to complete biomarker validation

Key: SA=secondary aim. PA=primary aim. AD=Alzheimer's disease. NP=not a priority. SOPs=standard operating procedures. Aβ=fibrillar β-amyloid. *With tracer florbetapir, flutemetamol, or florbetaben.

Study 2. Clinical validity of brain fluorodeoxyglucose positron emission tomography as a biomarker for Alzheimer's disease in the context of a structured 5-phase development framework.

Background Brain imaging with PET and FDG is a well-known and validated method for measuring cerebral glucose metabolism. The regional pattern of reduced brain metabolism is currently used as a diagnostic tool for the early and differential diagnosis of dementia and is included among the core biomarkers of neuronal degeneration and injury in different criteria for AD (7-8). However, its maturity for standard use in the clinical routine has never been systematically evaluated. The aim of the present article is to systematically review the evidence currently available on the use of FDG PET as a diagnostic biomarker for clinical and prodromal AD.

Methods This literature review followed the oncology framework (49) adapted to the field of neurodegenerative disorders, with the specific aim to differentially diagnose AD at the prodromal stage (50,53). The target population is subjects with mild cognitive impairment (MCI): the focus of the present effort is to evaluate the ability of biomarkers to identify AD at the MCI stage. The standard reference for diagnosis is AD neuropathology or development of incident AD dementia at 2-year follow-up. We considered only sporadic and not familial AD. A large literature is available on the use of FDG PET in AD and MCI. A Pubmed/Embase/Cochrane search performed on the 2.6.2015, with the following key words: “fluorodeoxyglucose” and “positron emission tomography” and (“Alzheimer” or “mild cognitive impairment”) and “diagnosis” yielded 553 results. Different strings were used to circumscribe the search specifically to the different aims and/or sub-aims of the phases. with the largest population (arbitrarily operationalized as a minimum of 50 subjects) were included; for the MCI conversion to dementia outcome, only studies with a minimum follow-up of 2 years for nonconverters were included. We checked the reference lists of any relevant

studies for additional studies and reviews of the literature have been subsequently included for answering specific sub-aims. In the absence of published data for specific aims and/or phases, the personal knowledge about abstract presentations was also reported. The diagnostic information of FDG PET studies resides in the comprehensive analysis of reduced and preserved metabolism in different regions. Some are typically affected in AD, with some specificity in different phases and subtypes. Other regions are spared, at least partly, in AD, and this information can be used as a differential diagnosis parameter with other types of dementia. In general, the severity of metabolic impairment and its topography correlates well with the severity of functional and/or neuropsychological impairment in different domains (memory, spatial processing, behavior, and so forth). The intrinsic complexity of this evaluation is the main reason for which the definition of the biomarker normality or abnormality and its threshold is indeed a debated matter, and a correct interpretation relies mostly on the integration of the information coming from all the differential impairment across all regions (54). We summarize in the following headings the main studies evaluating the different components of AD-related and noneAD-related patterns.

Hippocampus. The hippocampus is a very early site of pathological changes (mostly intraneuronal neurofibrillary changes) in most patients (55). The analysis of hippocampal metabolism is, however, limited by 2 main factors. First, the thickness of this anatomical structure is in the range of the spatial resolution of the majority of nondedicated PET scanners, mainly in case of atrophic hippocampi, as in AD. Second, the mesial temporal allocortex is physiologically hypometabolic, as compared with neocortical areas: these lower reference values reduce the dynamic range to document statistically significant reductions. Thus, the ability to identify hypometabolism on FDG PET images in this area depends heavily on the analysis methods and scanner resolution, and the occurrence of hippocampal hypometabolism in AD is variable in the literature (56, 57). Thus, hippocampal

hypometabolism is not a major hallmark in the detection of an AD pattern in individual reading.

Posterior cingulate cortex. The posterior cingulate cortex is a functional association crossroads, and its hypometabolism is at least partly due to disconnection mechanisms (56;58). It is the most sensitive marker on FDG PET and sufficient for considering FDG PET imaging suggestive of AD in MCI subjects (59). Given the high sensitivity, it has a lower specificity, and a mild impairment has been reported in young subjects at risk (ApoE epsilon4 carriers; 60).

Temporoparietal cortex. The temporoparietal association cortices (angular gyrus, precuneus, and so forth) are affected early in most cases of early onset AD, but only at later stages in most cases of late onset AD, and are a relevant part of the classical pattern for visual and automated reading of FDG PET, less sensitive than the posterior cingulate cortex alone but more strongly associated with disease severity and progression (61). Language deficits, which can present as the clinical syndrome of anomic aphasia, are associated with metabolic reduction in the dominant temporal lobe (62). The progressive involvement of brain regions in association with dementia severity in AD has been previously described by group analysis and has been modeled, and individual longitudinal trajectories have been described in individual patients with pathologically verified diagnosis (63).

Occipital cortex. Occipital metabolism is typically preserved in AD cases. However, prominent visuospatial deficits are typical for the AD subtype of posterior cortical atrophy, which exhibits prominent regional metabolic impairment in parieto-occipital visual association cortices (62). Occipital activity is also affected by acquisition conditions, namely eyes open or closed during uptake, which might be difficult to keep constant in patients with limited ability to cooperate. The existing literature indeed, uses both strategies.

Frontal cortex. Frontal metabolism may be reduced in AD in advanced cases, or in cases

with atypical behavioral, language, and dysexecutive presentation (64). The articles analyzed in this systematic review used hypometabolism in the mesial parietal regions, namely the precuneus, as FDG PET pattern for prodromal and clinical AD, variably associated with bilateral temporoparietal hypometabolism and hypometabolism in the temporal mesial regions, possibly associated with hypometabolism in frontal regions, less pronounced, and with relatively preserved metabolism elsewhere.

Non-AD-related cortical hypometabolism, marker of diseases other than AD for differential diagnosis Frontotemporal regions are typically hypometabolic in FTD, with various patterns typical for the various subtypes (65). Mesiofrontal metabolism is declining with age, also in controls (66). Occipital hypometabolism, especially when extending into primary visual cortex, is a typical sign of DLB cases (67). The sensorimotor cortex is typically preserved in neurodegenerative dementias, with the exception of corticobasal degeneration, which might involve primary motor areas of the affected hemisphere (68).

Results FDG PET has fully achieved phase 1 (rational for use) and most of phase 2 (ability to discriminate AD subjects from healthy controls or other forms of dementia) aims. Phase 3 aims (early detection ability) are partly achieved. Phase 4 studies (routine use in prodromal patients) are ongoing, and only preliminary results can be extrapolated from retrospective observations. Phase 5 studies (quantify impact and costs) have not been performed. The results are summarized in Table 2 and represented in Fig. 2.

Discussion and conclusions: The effort of the Geneva Task Force for the roadmap of AD biomarkers has been to summarize the evidence currently available for different biomarkers to properly validate their use in the perspective of a large and systematic use (50). The identification of aims and sub-aims only partially or not achieved should highlight areas needing further research and development. For this purpose, a validation scheme suggested for oncology biomarkers and adapted from drug development has been borrowed (49). This

exercise has required a relevant paradigm shift in translating a scheme developed for biomarkers to be used for screening in a healthy population (in oncology) to biomarkers used to diagnose prodromal AD in a population of patients (clinically diagnosed as MCI). Indeed, a major difference between the oncology field and the AD field exists, namely the fact that disease-modifying treatments exist in oncology, justifying a screening approach, while this is not the case in AD. The AD field has significantly evolved, in terms of understanding of disease characteristics and evolution, over the last years, also because of the increasing use of biomarkers, and diagnostic criteria have clearly integrated this knowledge (7-9). In the current clinical practice, biomarkers are helpful diagnostic tools to be used in selected cases for which the clinical diagnosis is problematic: atypical presentation, early age at onset, relevant comorbidities limiting the information that can be obtained by clinical and anamnestic evaluations (11). FDG PET, in particular, is a validated and well-recognized diagnostic tool; the fact that some sub-aims of phase III are not yet met indicates the route that needs to be covered to provide full validation based on a systematic procedure aimed to provide full demonstration that clinical utility overtakes the costs undertaken for examination. However, its current integration as a useful diagnostic marker in clinical practice for selected situations denotes that the solid analytical validation, as demonstrated in phase 1e2 studies guarantees already a precious and reliable diagnostic help for clinicians, already in the MCI phase (45). The main strength of FDG PET, as compared with other biomarkers, relies in its high predictive value for conversion, which provides useful insight into the risk of progression for individual patients, a highly relevant prognostic information for daily clinical use (24, 39-40). This unique feature of FDG PET will likely play a relevant role as soon as a disease-modifying drug becomes available to accurately select, among patients positive for pathophysiological markers, those at higher risk for progression over the following 2 years. On the other hand, also patients at low risk, i.e., with negative FDG PET

imaging, but already positive for amyloid markers could be the ideal candidates for preventive and/or therapeutic strategies, being functionally intact despite the presence of amyloid pathology. In the framework for biomarker evaluation proposed by Pepe et al. (49) for the oncology field and here adapted to the detection of prodromal AD, FDG PET, similarly to amyloid PET, medial temporal atrophy, and CSF assessment, has completed most of the aims and sub-aims of the first 3 phases (27-29). Large prospective studies of the clinical usefulness of a routine and systematic use in MCI (phase 4) and of its impact on health outcomes and costs (phase 5) are now needed.

Paper: Clinical validity of brain fluorodeoxyglucose positron emission tomography as a biomarker for Alzheimer's disease in the context of a structured 5-phase development framework. Garibotto V, Herholz K, Boccardi M, Picco A, Varrone A, Nordberg A, Nobili F, Ratib O; Geneva Task Force for the Roadmap of Alzheimer's Biomarkers. Neurobiol Aging. 2017 Apr;52:183-195.

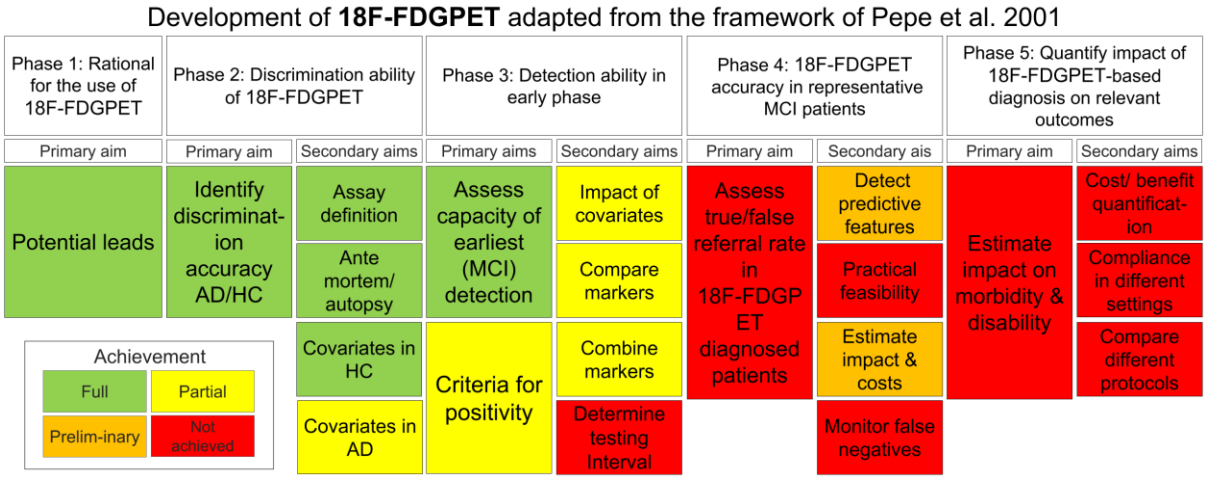


Figure 2: Synopsis of the maturity of 18F-FDGPET as borrowed from the oncology framework (Pepe et al., 2001).

Phase	Aim	Progress	Evidence in AD
(1) Preclinical exploratory studies	To identify leads for potentially useful biomarkers and prioritize identified leads.	Achieved	Glucose is the primary substrate of the synaptic function, which is affected by the neurodegeneration occurring in AD.
(2) Clinical assay development for clinical disease	Primary: to estimate TPR and FPR or ROC curve for the assay and to assess its ability to distinguish subjects with and without disease.	Achieved	Case-control studies show a good sensitivity and adequate specificity to discriminate patients and healthy controls, using pathology as the gold standard.
	Secondary 1: to optimize procedures for performing the assay and to assess the reproducibility of the assay within and between laboratories.	Achieved	Procedures for FDG PET acquisition and guidelines have been standardized (Guidelines of the European Association of Nuclear Medicine and of the Society of Nuclear Medicine and Molecular Imaging).
	Secondary 2: to determine the relationship between biomarker tissue measurements made on tissue (phase 1) and the biomarker measurements made on the noninvasive clinical specimen (phase 2).	Achieved	Glucose metabolism measured with the standard imaging protocol is a good approximation of full quantification of glucose consumption and correlates with neurodegenerative changes on pathology.
	Secondary 3: to assess factors (e.g., sex, age, and so forth), associated with biomarker status or level in control subjects. If such factors affect the biomarker, thresholds for test positivity may need to be defined separately for target subpopulations.	Achieved	Influence of age on glucose metabolism is present and known, no significant effect of gender. Influence of some medications is also known.
	Secondary 4: to assess factors associated with biomarker status or level in diseased subjects—in particular, disease characteristics.	Partly achieved	Different patterns of hypometabolism are associated with age at onset, ApoE4 positivity (with discordant results) and clinical variants (e.g., visual and language variant).
(3) Prospective longitudinal repository studies	Primary 1: to evaluate the capacity of the biomarker to detect the earliest disease stages.	Achieved	Results in MCI show moderate-to-high sensitivity and specificity for progression to AD; i.e., the ability to identify the disease in the earliest stage (prodromal AD) is confirmed.
	Primary 2: to define criteria for a biomarker positive test in preparation for phase 4.	Partly achieved	Quantitative analyses providing a cutoff for test positivity are available and widely tested, but no internationally recognized standard exists yet.
	Secondary 1: to explore the impact of covariates on the discriminatory abilities of the biomarker before clinical diagnosis.	Preliminary evidence	Preliminary data in AD patients on the impact of age at onset on diagnostic performance.
	Secondary 2: to compare markers with a view to selecting those that are most promising.	Partly achieved	Preliminary studies with discordant results (possibly due to the type of analysis used rather than biomarker performance).
	Secondary 3: to develop algorithms for positivity based on combinations of markers.	Partly achieved	Preliminary evidence on association of CSF Aβ42, hippocampal atrophy, and DaTSCAN to FDG PET to increase diagnostic accuracy
	Secondary 4: to determine a biomarker testing interval for phase 4 if repeated testing is of interest.	Preliminary evidence	
(4) Prospective diagnostic studies	Primary: to determine the operating characteristics of the biomarker-based test in a relevant population by determining the detection rate and the false referral rate. Studies at this stage involve testing people and lead to diagnosis and treatment.	Not achieved	
	Secondary 1: to describe the characteristics of disease detected by the biomarker test—in particular, with regard to the potential benefit incurred by early detection.	Preliminary evidence	Early detection by FDG PET imaging might allow earlier treatment and change treatment strategy, mainly in cases of unclear and/or atypical dementia.
	Secondary 2: to assess the practical feasibility of implementing the case finding program and compliance of test-positive subjects with work-up and treatment recommendations.	Not achieved	
	Secondary 3: to make preliminary assessments of the effects of biomarker testing on costs and mortality associated with the disease.	Preliminary evidence	Preliminary data show that the improved diagnostic accuracy by FDG PET is cost-effective in dementia: no data on prodromal disease and in a longitudinally designed study are available yet.
	Secondary 4: to monitor disease occurring clinically but not detected by the biomarker testing protocol.	Not achieved	
(5) Disease control studies	Primary: to estimate the reductions in disease-associated mortality, morbidity, and disability afforded by biomarker testing.	Not achieved	
	Secondary 1: to obtain information about the costs of biomarker testing and treatment and the cost per life saved or per quality-adjusted life year.	Not achieved	
	Secondary 2: to evaluate compliance with testing and work-up in a diverse range of settings.	Not achieved	
	Secondary 3: to compare different biomarker testing protocols and/or to compare different approaches to treating test-positive subjects in regard to effects on mortality and costs.	Not achieved	

Table 2. Summary of biomarker results according to the 5-phase structure. Key: AD, Alzheimer's disease; CSF, cerebrospinal fluid; FDG PET, fluorodeoxyglucose positron emission tomography; FPR, false-positive rate; TPR, true-positive rate; MCI, mild cognitive impairment.

Study 3. Early identification of MCI converting to AD: a FDG PET study

Background: One of the main clinical issues in a memory clinic is to predict whether a MCI will convert to dementia. MCI patients convert at an average rate of 10–17% per year (69-71), yet a substantial proportion of them remain stable or improve after some years (72). These 'nonconverter' MCI patients (nc-MCI) represent 20% to 40% of the MCI cohort (73) and are affected by a variety of conditions that may mimic a neurodegenerative disease (74). Structural and functional neuroimaging have been used to discriminate normal subjects from MCI patients who convert to AD (MCI-AD), with relatively high accuracy (75-77). On the contrary, distinguishing MCI-AD from nc-MCI patients remains a major challenge. Among the AD biomarkers, FDG PET has shown accuracies in predicting progression of MCI to AD ranging between 70% and 83% (70, 78, 79). This lack of consistency may be partly caused by limited patient follow-up. This specific limitation could hamper the selection of appropriate conversion-related regions, since nc-MCI may also show hypometabolism in critical areas (80). Applying multivariate analysis techniques based on principal component analysis (81) and independent component analysis (82), our group has recently found progressive alterations from normal ageing (NA) to MCI and AD. Our studies also revealed accuracies close to 90% for FDG PET in discriminating patients with nc-MCI from MCI-AD and AD. The aims of this study were to: (1) assess the accuracy of an analysis based on volumes of interest (VOIs) in discriminating between nc-MCI and AD-MCI patients, and (2) identify the regions that mostly contribute to the differentiation of the two groups. For reliable discrimination, the classification was based on a follow-up of at least 5 years after the baseline PET.

Materials and methods 164 subjects were divided into three groups: 42 normal elderly subjects (NA), 27 nc-MCI; mean follow-up 7.5 ± 1.5 years, range 5.0–9.8 years, and 95 MCI-AD; mean time to conversion 1.8 ± 1.1 years, range 0.4–5.0 years).We chose not to consider a-MCI patients who converted to dementias other than AD. MCI diagnosis (71)

included pure amnestic or multidomain MCI (83). The controls were healthy volunteers carefully evaluated by clinical examination and by their general medical history. Baseline evaluation for all subjects included morphological (MRI) and functional (FDG PET) neuroimaging and extensive neuropsychological examination. (83). A 15-item Geriatric Depression Scale (GDS) score of ≤10 was required for inclusion, while MRI evidence for vascular cognitive impairment was an exclusion criterion (84). The 27 nc-MCI patients who were followed up for a minimum of 5 years received a different diagnosis at the last follow-up. Of the control subjects, 25 (60%) were followed up after 12 to 107 months (mean 41.8 ± 31 months), with confirmation of their healthy status. FDG PET images were preprocessed using Statistical Parametric Mapping (SPM8) with a customized brain FDG PET template optimized for dementia patients (85) FDG uptake values were calculated in 45 anatomical VOIs in each hemisphere, as defined by the Automated Anatomical Labeling Atlas (86) and analyzed using a Matlab-based script created in-house that automatically processed the mean FDG. uptake value from each of the VOIs bilaterally. The extracted values were then normalized in each subject to the average intensity of the cerebellar VOIs on the basis that the cerebellum is poorly affected by the AD pathological process. To decrease the number of variables, the number of VOIs was reduced by merging regions with similar anatomo functional characteristics into 13 meta-VOIs in each hemisphere. One-way analysis of variance was used to evaluate the significance of differences between neuropsychological tests. To determine the value of meta-VOI-based FDG uptake in predicting the possible onset of AD, we applied a support vector machine (SVM) model using age-corrected baseline data to discriminate between nc-MCI and MCI-AD patients. The SVM model was implemented with a radial basis function (Gaussian) kernel (87) with a constant scale factor derived from a previous study (77). A stepwise selection procedure was applied to identify the sets of regions that provided the highest discrimination. Subjects were divided into 21 subgroups (21-fold

cross-validation), each including a proportional share of subjects from the two groups (about 5%). The training and testing procedure were repeated 21 times. For each subgroup, the SVM model was fitted to all remaining subjects and then applied to each subject in the subgroup to produce a score measuring group membership, and a consequent classification. Thus, an individual score was produced for each investigated subject based on a virtually independent training set, and this enabled a receiver operating characteristic (ROC) curve to be built. To evaluate the accuracy, sensitivity and specificity of the method, a cut-off value was chosen as the minimum the distance from the upper left corner of the ROC curve (where specificity = sensitivity = 1). Accuracy, sensitivity, specificity and the area under the ROC curve (AUC) were determined along with their confidence intervals (CIs). The Wald interval, with exact binomial probabilities, was used for sensitivity, specificity and accuracy (88), and the CIs for the ROC AUCs were estimated using the bootstrap method described by Qin and Hotilovac (89). Statistical analyses were performed using the Statistics Toolbox of Matlab R2015b (MathWorks, Natick, MA).

Results Only two neuropsychological tests (Rey Auditory Verbal Learning Test immediate total recall and Rey Auditory Verbal Learning Test delayed recall) showed significant differences between the two MCI groups ($p < 0.05$ and $p < 0.001$, respectively). Repeated measures analysis of variance showed highly significant differences among the mean values associated with all the effects: group ($F_{2,161}$ 13.65, $p < 0.0001$), region ($F_{25,4025}$ 801.84, $p < 0.0001$) and their interaction ($F_{50,4025}$ 3.54, $p < 0.0001$). Post hoc analysis showed significantly lower mean values in the MCI-AD group than in the two other groups. The pattern of regional mean values in the nc-MCI group was close to that in the NA group in most regions and was intermediate between the patterns in the latter group and the MCI-AD group. Most regions, including the parahippocampal gyrus/amygdala/hippocampus/insula, the cuneus/fusiform gyrus/precuneus, the posterior

cingulate gyrus, and the parietal and temporal lobes, showed significantly lower values in the MCI-AD group than in the NA group. In some of these regions, a similar decreasing trend was also found with respect to the nc-MCI group and the difference survived Bonferroni correction in the left cuneus/fusiform gyrus/precuneus, the left posterior cingulate gyrus and the right temporal lobe. Application of the SVM model resulted in a fair partition between MCI-AD and nc-MCI patients with a set of only three meta-VOIs (Fig. 3.1) that, after cross-validation, was able to discriminate the two groups with 82.0% accuracy (CI 75.2–88.8%), 81.1% sensitivity (detection of MCI-AD; CI 73.2–88.9%) and 85.2% specificity (CI 71.8–98.6%), and an AUC of 0.834 (CI 0.742–0.900; Fig. 2). Using 13 meta-VOIs (listed in fig 3.3) the model was able to discriminate between AD-MCI and nc-MCI patients with 88.5% accuracy (CI 82.9–94.2%), 87.4% sensitivity (detection of AD-MCI; CI 80.7–94.1%) and 92.6% specificity (detection of nc-MCI; CI 82.7–100.0%), and an AUC of 0.911 (CI 0.835–0.957; Fig. 3.2). Among these meta-VOIs, the greatest decreases in mean FDG uptake values in the MCI-AD patients with respect to the nc-MCI patients were found in the right temporal and parietal lobes, and in the cuneus/fusiform gyrus/precuneus of both hemispheres. The difference in the AUCs between the two models (three meta-VOIs and 13 meta-VOIs) was of borderline significance in the Hanley and McNeil test ($z = 1.7$, $p = 0.045$; Fig. 3.2).

Discussion and conclusions: it is important to identify the early hallmarks of conversion of MCI to AD. The use of FDG PET as a tool to predict the progression of neuronal injury is based on its stronger association with cognitive deterioration. The most relevant result of this study is the identification of meta-VOIs with impaired metabolism in patients with MCI converting to AD. These areas were impaired not only compared with uptake in NA patients, but also with that in nc-MCI patients, whose values were similar to NA (Table 2). Metabolic data from brain regions of 122 MCI patients were segmented using a freely available anatomical atlas and extracted by a Matlab-based script developed in-house that

pooled them into 13 meta-VOIs, bilaterally, which were then analyzed using the SVM. This process could be more easily applicable in clinical practice than others (91) and has reported an overall accuracy of 89% and a well-balanced sensitivity and specificity. In fact, this method correctly classified 87% of MCI-AD and 93% of patients nc-MCI. Furthermore, we found significantly different metabolic rates in most of the association cortices between MCI-AD patients and NA subjects, while the differences between MCI-AD and nc-MCI patients were focused on a smaller set of regions, where values in the nc-MCI patients were not significantly different from those in NA subjects. Regions surviving subtraction of the differences between the nc-MCI patients and NA subjects were the most robust predictors of conversion; these regions included the parietal lobe, the posterior cingulate gyrus, the temporal pole and lobe, and the cuneus/fusiform gyrus/precuneus (Table 2). Differences in metabolic values in other regions, including the medial temporal lobe (parahippocampal gyrus/amygdala/hippocampus/insula) and the anterior cingulate gyrus, were not sufficient to discriminate between MCI-AD and nc-MCI patients. This finding is especially useful in the clinical context, since other conditions leading to MCI, including depression (92) and vascular cognitive impairment (93), might affect brain metabolism in these areas. It is worth noting that the putamen/pallidum/caudate was among the regions selected for SVM classification. This region is generally considered less affected by the pathological AD process, and in this study did not show significant differences between nc-MCI and MCI-AD patients (Figure 3.2). The presence of relatively spared regions has been previously observed (94) and the identification of such regions probably contributes to discrimination in that the differences between affected and spared regions are highlighted and are apparent in the early and intermediate stages of the pathological process. Some of the strengths of this study were the very homogeneous method of investigation performed by the same clinical group and the extended follow-up (minimum 5 years) in nc-MCI patients, which, to the best of our

knowledge, exceeds that in previous studies. Considering the high accuracy obtained by the present meta-VOI-based SVM analysis of a highly homogeneous dataset, a further step for its application as an automatic tool in the clinical setting should be its fitting and validation in a larger and multicentre dataset. This study not included neurodegenerative dementing disorders other than AD. This could be a selection bias that restricts the diagnostic value of our results since these patients might have been missed by FDG PET, or wrongly included in one of the three groups. In conclusion, in MCI patients not converting to AD within a minimum follow-up time of 5 years and MCI patients converting within 5 years, baseline FDG PET and volume-based analysis identified those who converted with an accuracy of 89%. Indeed, there is an urgent need to harmonize the interpretation and reporting of FDG PET scans (26), as the method adopted for analysis can be as important as the choice of the biomarker itself (95), providing it is also tested in a-MCI patients later developing dementia other than AD.

Paper: Pagani M, Nobili F, Morbelli S, Arnaldi D, Giuliani A, Öberg J, Girtler N, Brugnolo A, Picco A, Bauckneht M, Piva R, Chincarini A, Sambuceti G, Jonsson C, De Carli F. Early identification of MCI converting to AD: a FDG PET study. Eur J Nucl Med Mol Imaging. 2017 Nov;44(12):2042-2052

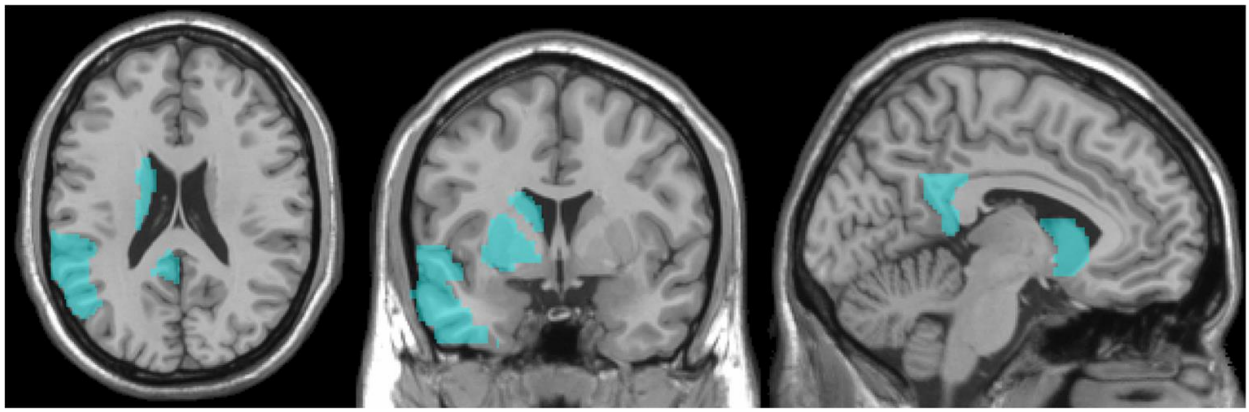


Fig.3.1

Topographic representation of the three meta-VOIs set in the left hemisphere with the best discrimination ability superimposed on the Montreal Neurological Institute template in the transverse (left), coronal (centre) and sagittal (right) views

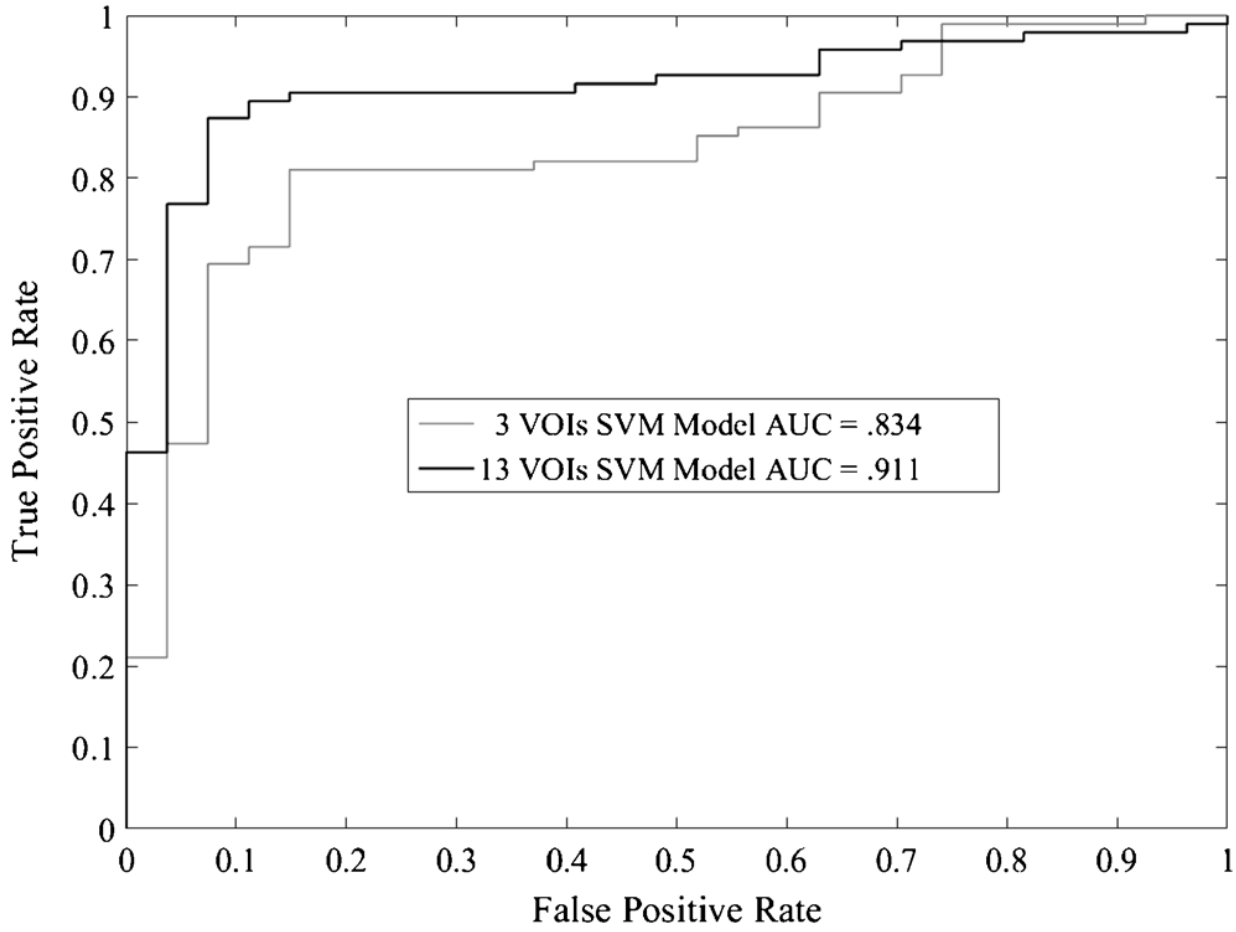


Fig. 3.2 Receiver operating characteristic curves showing the accuracy of the SVM model in discriminating between non-converter MCI patients and MCI patients who subsequently converted to dementia. The grey curve was obtained using three selected regions; the black curve was obtained using 13 selected regions

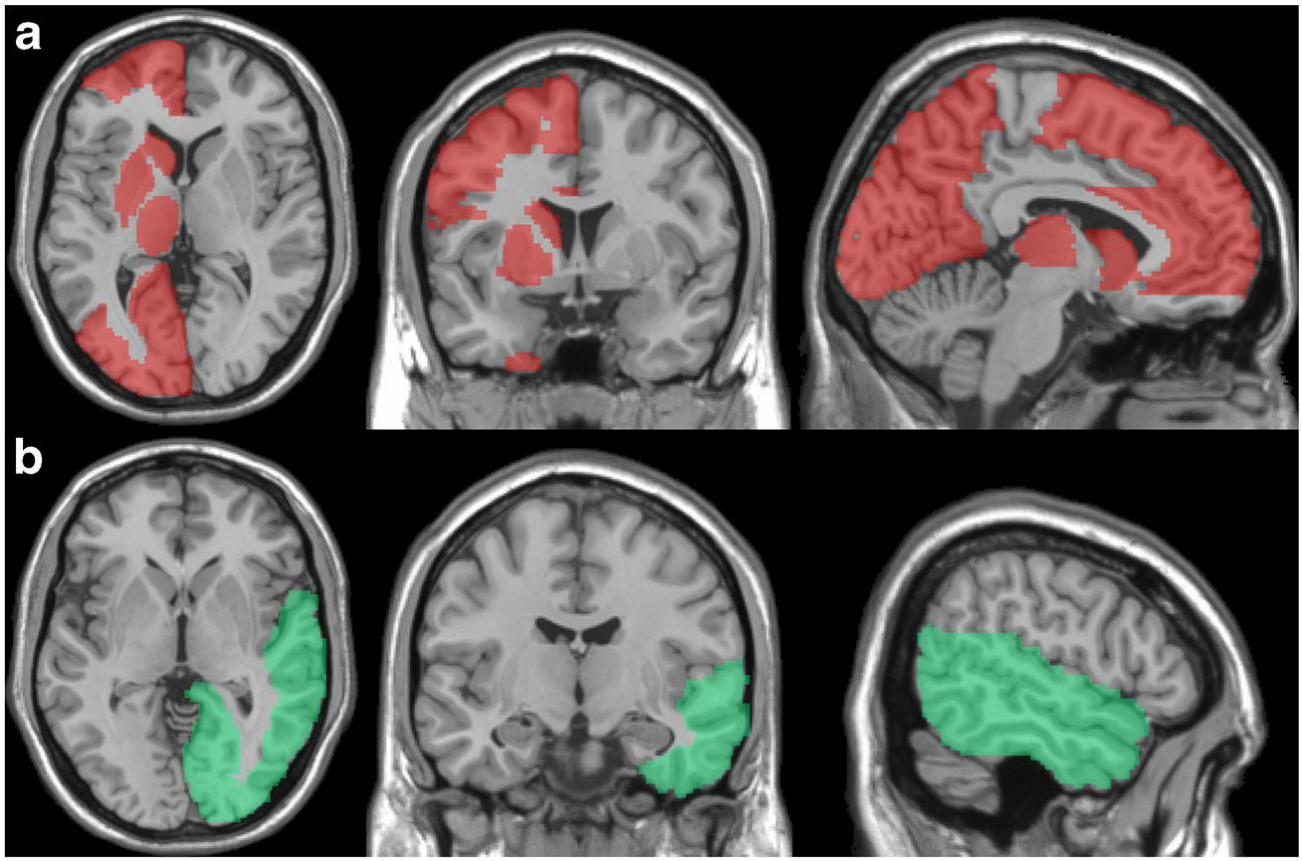


Figure 3.3 Topographic representation of the 13 meta-VOI sets with the best discrimination ability superimposed on the Montreal Neurological Institute template. a Left hemisphere set (red. Left: transverse view, centre: coronal view, right: sagittal view). B Right hemisphere set (green. Left:transverse view, centre: coronal view, right: sagittal view). Composition of each meta-VOI were: occipital cortex and cuneus/fusiform gyrus/ precuneus bilaterally, thalamus, putamen/pallidum/caudate and orbitofrontal and frontal cortices, anterior cingulate gyrus, and the postcentral gyrus/precentral gyrus/supplementary motor area on the left side, and the right temporal pole, and parietal and temporal lobes (Fig. 3.3).

Study 4. 18F–FDG PET diagnostic and prognostic patterns do not overlap in Alzheimer's disease patients at the mild cognitive impairment stage

Introduction. The International Working Group Criteria 2 defined FDG PET as progression biomarkers, because of its potentially usefulness to assess downstream neurodegeneration, disease progression, and to intercept time to disease milestones (7). The identification of predictors of time to disease milestones such clinical conversion to dementia in MCI due to AD is pivotal either for patients' follow-up and counseling than for its potential utility as surrogate endpoints in clinical trials. While a large body of literature has been devoted to assess the value of FDG PET in the prediction of further cognitive decline in MCI for diagnostic purposes (early identification of MCI due to AD), fewer studies have been dedicated to investigate the accuracy of FDG PET in estimating the rate of progression toward dementia in AD patients undergoing FDG PET at the stage of MCI (45,82,96). More importantly, while the AD-typical pattern of hypometabolism has been consistently confirmed in patients with MCI due to AD (MCIAD), the topographical localization of a FDG-PET 'progression' pattern has not yet been topographically located. In this study we aimed to identify the cortical regions where metabolic impairment may help predicting the time to clinical conversion to dementia in patients with MCIAD.

Methods We selected a series of consecutive outpatients with these characteristics: (1) to be evaluated for the first time for a suspicion of MCIAD; (2) to undergo brain FDG PET at baseline; and (3) to be followed up at least until clinical conversion to AD dementia with a regular control visit allowing the definition of their conversion time with a degree of uncertainty lower than 6 months. The diagnosis of MCI followed Petersen's criteria (83). The control subjects were 48 healthy volunteers (CTR) with similar age, gender and educational as patients. Their healthy condition (including normal MMSE and CDR score 0) was carefully checked with the same exclusion criteria as for patients, except for cognitive complaints.

These subjects underwent the same neuropsychological battery as patients, FDG-PET, and MRI. FDG-PET was acquired according to the guidelines of the European Association of Nuclear Medicine (97). Preprocessing of FDG-PET images was performed using the default choice of SPM8 stand-alone version (Wellcome Department of Cognitive Neurology, London, UK). However, the H215O SPM-default template was replaced by a dementia-optimized brain FDG-PET template as described by Della Rosa and colleagues (85). The spatially normalized set of images was then smoothed with an 8-mm isotropic Gaussian filter. PET scans both in CTR and in MCI-AD patients were qualitatively evaluated in consensus by three experts in nuclear neurology (S.M., M.B. and F.N.). All CTR subjects revealed a symmetrical and normal brain distribution of 18F–FDG. The AD typical pattern was present in 74 (90%) patients. Concomitant hypometabolism in occipital or frontal cortex was present in some of these patients showing a more extended and severe cortical hypometabolism. The following analyses were performed in SPM8 (98) using the cerebellum as reference area for the intensity normalization (99): 1. MCI-AD were compared with CTR by means of two sample t test design to find AD typical pattern (age, gender and education were used as nuisance). The resulting cluster of hypometabolism is from now on referred in the text as Diagnostic Pattern; 2. Multiple regression analysis was performed in MCI-AD to evaluate the correlation between conversion time and metabolism (age, gender, education, and baseline MMSE score were included as nuisance). The resulting cluster of correlation is from now on referred in the text as prognostic pattern; 3. MCIAD group was divided into early and late converters according to their conversion time with respect to the mean time of conversion of the entire group. Then by means of a two-sample t test, early and late converters subgroups had compared each other in both directions (age, gender and education were used as nuisance). For all SPM group analyses, significance threshold was set at $p < 0.05$ False-Discovery-Rate- corrected both at peak and at cluster level. Each MCI-AD patient was then individually compared with the CTR

group in a SPM single-subject analysis to assess the presence of hypometabolism within at least one of the BA included in either Diagnostic or Prognostic patterns, or both. In this frame the use of SPM to identify the presence of hypometabolism within these regions allowed an objective, observer-independent evaluation. This analysis was carried out using the same SPM rules as in group comparison between MCI-AD and CTR, apart from using an uncorrected $p < 0.001$ at peak level, to avoid type II errors (100). Time to conversion to AD-dementia was estimated using the Kaplan-Meier method and curves were compared by means of Log-rank as well as with the Breslow and Tarone-Ware tests. Statistical significance level was set at $p < 0.05$. To prevent overfitting and evaluate the stability of the topography of the prognostic pattern, the entire procedure, from the identification of the prognostic pattern to the generation of Kaplan Meier curves, was repeated with a leave-one-out cross-validation approach. The statistical analysis was conducted using SPSS version 17.

Results Eighty-two consecutive MCI subjects matched the study criteria (age: 75.3 ± 6.7 years; 55 females, 27 males; baseline-MMSE 26.9 ± 1.9;). They converted to AD dementia 6 to 43 months after the baseline visit (mean: 22.7 ± 12.2). Patients were then defined as early converters, if they clinically converted to AD Dementia within a follow up time of 22.7 months (n = 57), and late converters, if they converted after this cut off (n = 25). Comparison between MCI-AD and CTR highlighted the expected cluster of hypometabolism involving wide regions of the posterior parietal cortex, middle and superior occipital gyri, the precuneus and posterior cingulate cortex in both hemispheres (BA 7, 18, 19, 30, 31 and 40) and the left superior temporal gyrus in the left hemisphere (BA 22). (Fig. 1A and Table 2A). Furthermore, time to conversion was significantly and directly correlated with metabolic level in the right middle and inferior temporal gyri as well as in the fusiform gyrus (BA 20, 21 and 38). Accordingly, a faster progression rate was correlated with a lower metabolism in these cortical regions. The correlation (Fig. 1B and Table 2B) survived even after adjusting for the severity

of hypometabolism in the diagnostic pattern and was confirmed in the context of a leave-one-out cross validation. Early MCIAD converters resulted to have a significant relative hypometabolism in comparison late converters. The diagnostic pattern was identified in 61/82 patients (74.4%) while the prognostic pattern was found in 29/82 patients (35.4%), eight patients showing both the two patterns. All the eight patients without clear hypometabolism on visual analysis (see above) failed to show significant hypometabolism in either the diagnostic or the prognostic pattern when evaluated by means of single-subjects SPM approach. Kaplan-Meier analysis curves were not significantly different between patients with either presence or absence of the diagnostic pattern (median value: 15 months vs 14 months, respectively, p = n.s.). By contrast, the curves yielded a significant difference between patients with either presence or absence of the prognostic pattern (median value: 9 versus 19 months; Log rank $p < 0.02$, Breslow test: $p < 0.003$, Tarone-Ware test: $p < 0.007$). Finally, we explored the difference between the conversion time of the patients showing relative hypometabolism within all the BA included in the prognostic pattern (n = 12) and the other patients. The median conversion time was even more significantly shorter in the former group (8 versus 19 months $p < 0.001$) and all the twelve patients converted to AD dementia within two years (Fig. 3). In the leave-one-out cross validation, the prognostic pattern was highlighted in 37/82 patients. This different division resulted in a slight loss of significance with respect to the original model but the subgroup of patients showing hypometabolism in the prognostic pattern had still a significantly shorter progression rate with respect to the other subgroup (median value: 13 versus 19 months; Log rank $p < 0.02$, Breslow test: $p < 0.007$, Tarone-Ware test: $p < 0.011$). Kaplan Meyer analysis is reported in Fig. 4.

Discussion. The present study identifies for the first time that metabolism in the middle and inferior temporal cortex is correlated with timing of conversion to AD dementia in a naturalist population of MCIAD. Interestingly, this finding was independent of age,

education, baseline MMSE score, and even of the severity of hypometabolism in the posterior diagnostic pattern. Therefore, a more prominent hypometabolism in different cortical regions may represent a further source of heterogeneity in MCI-AD since the earliest stage of disease. In line with this evidence, the first Tau PET studies in AD patients confirm the biological heterogeneity of AD and further support the present results (101, 102). Annual changes in Tau tracer binding were prominent and significantly elevated in AD patients with respect to controls, specifically the middle and inferior temporal gyri and in fusiform gyrus (102). By contrast, the accurate and early diagnostic relevance of the posterior diagnostic FDG PET pattern would render it less sensitive to further biological progression of the disease by suffering of a sort of floor effect, not shared by those areas with less severe hypometabolism. The expression of both the diagnostic and prognostic pattern in MCI patients not converting to dementia (nc-MCI) and the relative weight on the two patterns in nc-MCI was outside the aims of the present study. However, it is worthwhile to mention that in a previous study based on a larger group of MCI-AD including the present cohort of patients, we demonstrated that the pattern of regional FDG uptake values in the nc-MCI group is close to the CTR group value in most cortical regions (39). Furthermore, in the same study, a small set of VOIs including posterior cingulate gyrus and temporal lobe already allowed partition with respect to MCI-AD, thus confirming the relevance of both patterns as biomarkers of the disease. Finally, it is worthwhile to underline that previous studies have located the capability of FDG PET of following AD clinical progression outside the AD-typical posterior pattern (103). In some other studies, a potential role in tracking disease progression has been proposed for the frontal cortex metabolism/function especially given its relevance in executive function, which is essential for the performance of IADL (42, 80, 103). However, the present one is the first study specifically correlating conversion time and whole brain metabolism without topographical a priori hypotheses while previous studies have been carried out by means of

pre-selected region of interest analyses (ROIs) (104). Finally, very recently another study has faced the issue of rate of functional decline in MCI-AD belonging to the ADNI database (105). That study is based upon a dementia prognosis index (DPI), derived from a ratio between the FDG uptake values in several ROIs known to be hypometabolic in AD and regions known to be stable. The authors demonstrated that DPI powerfully predicted a rate of functional decline among MCI patients as well as a rate of cognitive decline on MMSE. The largest and most significant clusters predictive of cognitive decline in MCI populations over time included the posterior cingulate cortex, right parietotemporal cortex, and left parietotemporal cortex. However, a further data-driven modified version of the DPI that additionally included the bilateral inferior temporal cortex was able to predict rate of functional decline among MCI patients even more powerfully, in keeping with our results. We acknowledge some limitations of this study. First, since APOE genotype was available in a minority of patients, we could not test the possible influence of APOE on the results. However, the presence of APOE ε4 genotype and its correlation with a faster progression of the disease has been previously linked with the posterior parietal cortex and not with the inferior and middle temporal cortex (106). Similarly, we lack an amyloidosis biomarker in most of the patients, and thus, we did not include it in the analysis. Therefore, the confirmation of AD was based on the results of neuropsychology, MRI and FDG-PET examinations at baseline and then clinically confirmed at the time of the clinical diagnosis of dementia of Alzheimer's type and in the further clinical follow-up. Therefore, we believe the risk of misdiagnosis has been minimized. Finally, the present results need to be confirmed in a larger group of AD patients, and, in this framework, we cannot exclude that the specific involvement of the right (rather than left) hemisphere could have been partially influenced by the most affected hemisphere in this group of AD patients. In fact, while the topographical pattern of temporoparietal hypometabolism is consistent in MCIAD, metabolic asymmetries with more prominent hypometabolism in one

or the other hemisphere are frequently present in FDG brain distribution in AD patients, especially early in the course of the disease. Moreover, left temporal hypometabolism was here highlighted within the diagnostic pattern, while the right temporal hypometabolism was not, and was instead included in the prognostic pattern. At an individual level, this might suggest that a symmetric temporal hypometabolism would stand for a more advanced disease stage (and higher risk of conversion) than an asymmetric temporal lobe hypometabolism. Finally, given the aims of the present study we choose not to correct PET results for partial volume effect (PVE), as the underlying atrophy is a further sign of the neurodegeneration process. This lack of correction might have further influenced the results and should be considered in their interpretation and transfer to a single-patient basis.

Conclusion The present findings demonstrated that the relevance of FDG-PET as a progression biomarker and its capability to predict speed of conversion to dementia is topographically located in the right middle and inferior temporal cortex and not in the typical posterior regions of AD signature in a naturalist population of MCI-AD patients. The present results obtained by an automated and observer-independent method can be translated to an individual patient level in the clinical setting and might be a clue in searching for a region to be monitored during interventional trials.

Paper: Morbelli S, Bauckneht M, Arnaldi D, **Picco A**, Pardini M, Brugnolo A, Buschiazzo A, Pagani M, Girtler N, Nieri A, Chincarini A, De Carli F, Sambuceti G, Nobili F. 18F-FDG PET diagnostic and prognostic patterns do not overlap in Alzheimer's disease (AD) patients at the mild cognitive impairment (MCI) stage. Eur J Nucl Med Mol Imaging. 2017 Nov;44(12):2073-2083. doi: 10.1007/s00259-017-3790-5. Epub 2017 Aug 7.

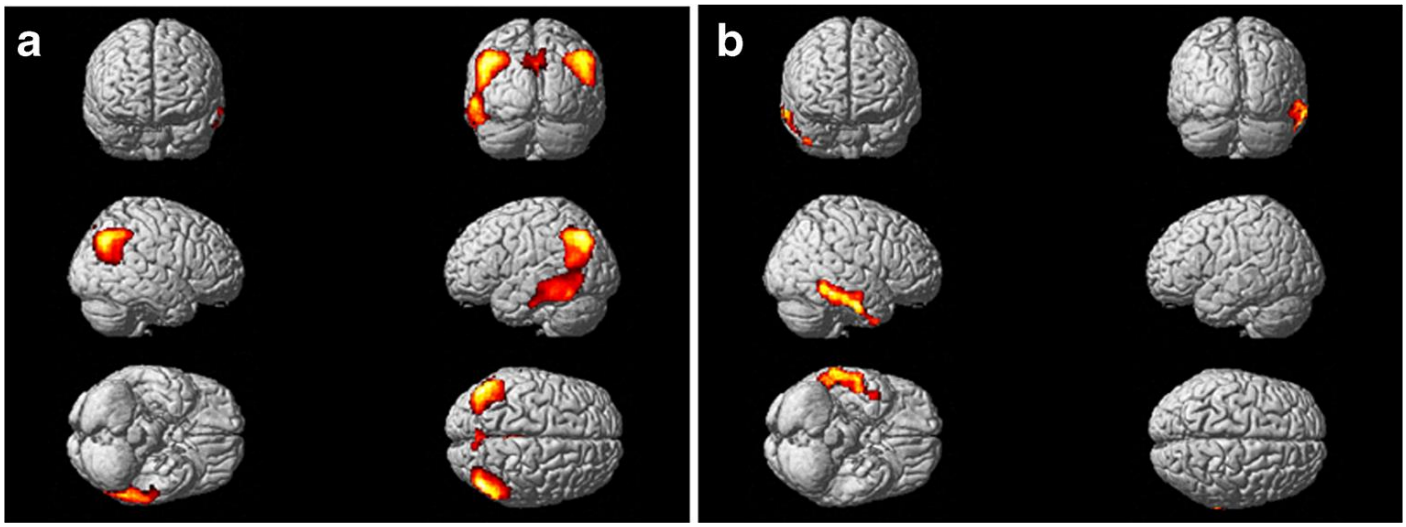


Fig. 1 Diagnostic and Prognostic patterns a: Comparison between healthy controls and MCI-AD patients (diagnostic pattern). b: Correlation between time to conversion to AD-dementia and whole brain metabolism in MCI-AD (prognostic pattern).

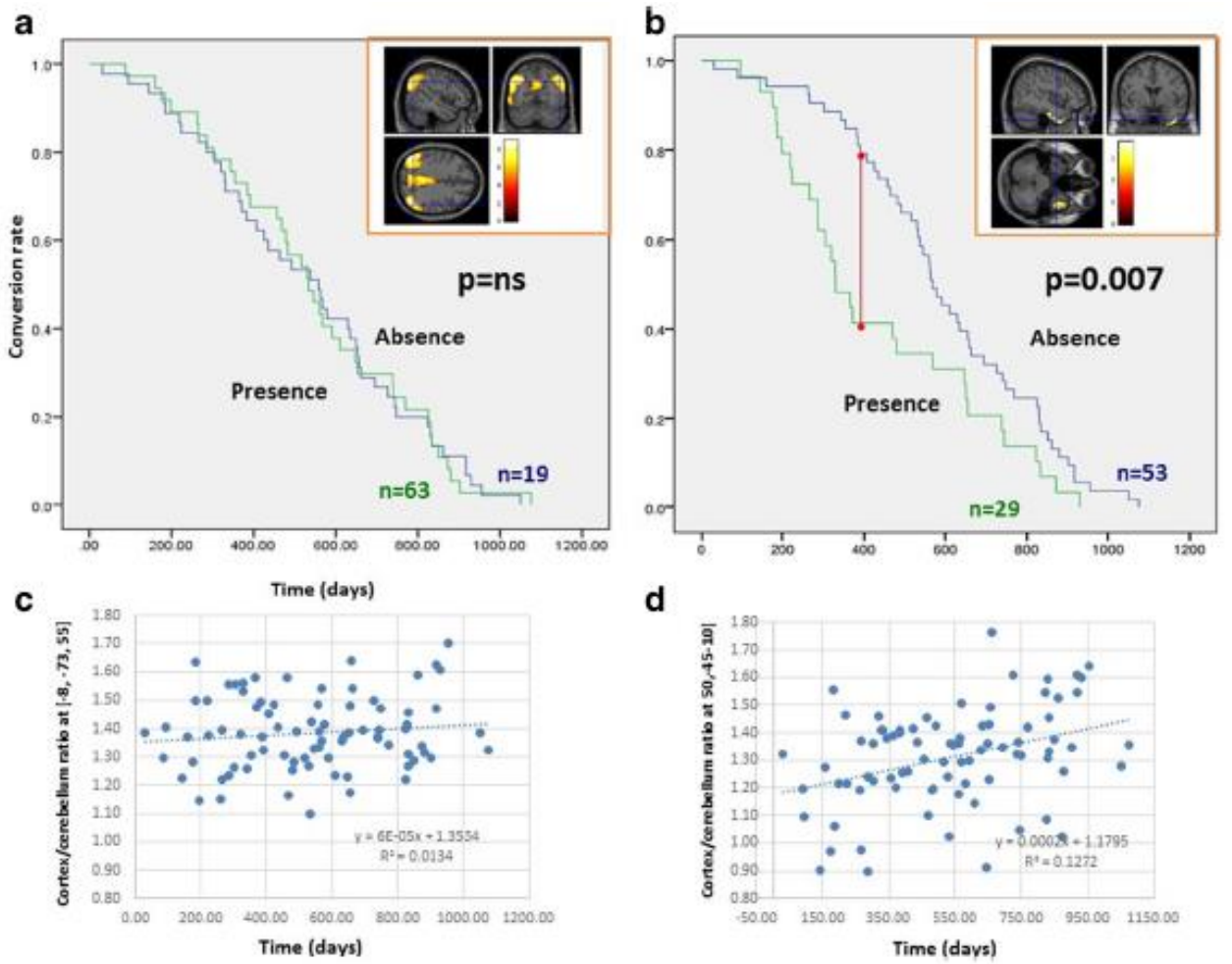


Fig. 2 Kaplan Meyer curves showing the conversion rate of MCI-AD patients. Conversion rate according to the presence/absence of at least one Brodmann area included in the diagnostic (A) and then in the prognostic (B) patterns in the SPM single subject analysis. The curves were not significantly different in patients with either presence or absence of the diagnostic pattern (Panel A) while a significant difference was highlighted between patients with either presence or absence of the prognostic pattern (Panel B). Panel C and D show the correspondent plots as generated by SPM expressing the correlation between brain metabolism in global maxima (voxel of highest Z-score in SPM) on one side and conversion time in the whole group of 82 MCI-AD

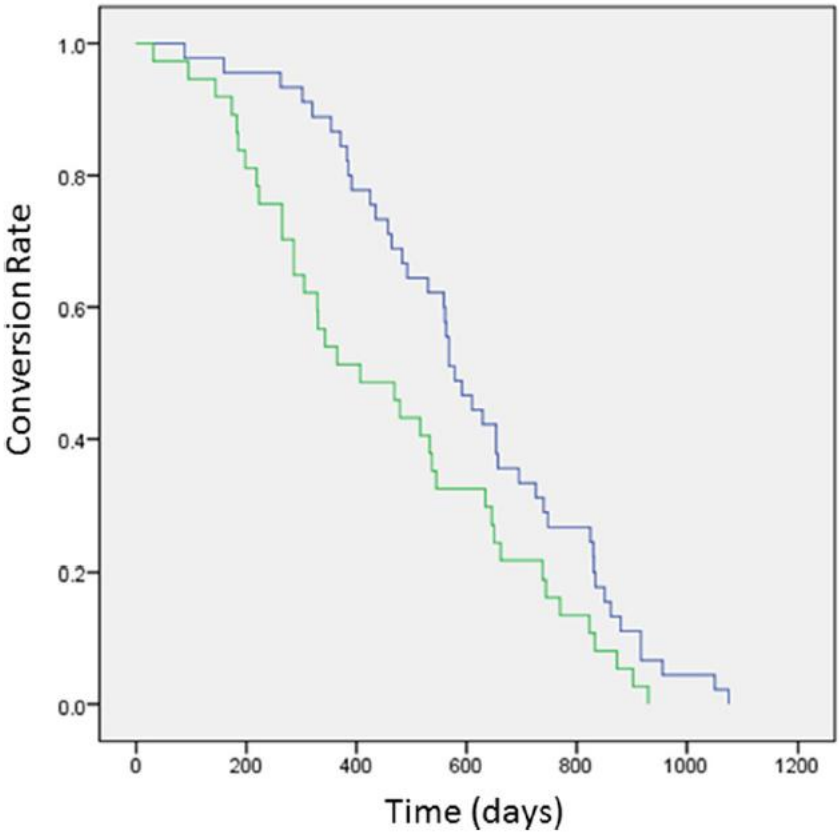


Fig. 4 Kaplan Meyer Curves showing the conversion rate of MCI-AD patients according to the presence/absence prognostic pattern after the leave-one-out cross validation. This different division resulted in slight loss of significance with respect to the original model, but the subgroup of patients showing hypometabolism in the prognostic pattern had still significantly shorter progression rate with respect to the other subgroup.

Study 5 (in progress): 18F-FDG-PET in Depressive Pseudodementia

Background. Depressive pseudodementia (DPD) is a relatively uncommon syndrome with cognitive impairment associated with depression mimicking symptoms of neurodegenerative dementia. This clinical entity was firstly described by Kiloh in 1961 (107) and consists of depressed patients who show poor cognitive performance based on poor attention, concentration not due to organic disorder (108). The importance of distinguishing primary dementing processes from functional disorders has been highlighted time and again because DPD is potentially reversible (109). On the other hand, clinical-neuropsychological diagnosis is often hampered by presence of depressive symptoms in neurodegenerative disorders, such Alzheimer's disease (AD). In fact, depression and cognitive deficit are often associated (108): 32% of patients with Mild Cognitive Impairment (MCI) suffer of depression (110). Depressed patients show moderate cognitive deficits (i.e. executive function, memory and attention), and some cognitive deficits, especially executive function and attention, could persist even after remission of depressive state (111). Furthermore, depression is a risk factor for Alzheimer's disease and mild cognitive impairment (112). Still, there is a lack of knowledge on the relationship between depression and dementia (113) because the role of depression and depressive symptoms in developing dementia is unclear: they can share the same risk factors, or they can coexist: depression can be a MCI prodromal symptom, a psychological reaction to mild degree of cognitive decline, or incident depression can unmask clinical manifestation of MCI in individuals with limited cognitive reserve.

DPD diagnosis is due by negativity of AD and other disease-specific biomarkers, and the clinical follow up can confirm the validity of the first diagnostic assessment. FDG-PET could have a main role in the early differential diagnosis from DPD from AD and other neurodegenerative brain disorders, since a normal scan virtually excludes dementia due to a

neurodegenerative disease, but specific studies are lacking (48). The aim of our study was to compared brain FDG-PET among DPD patients, patients with early AD and normal subjects, in order to better characterize brain metabolic pattern of DPD.

Methods. Twenty-eight consecutive outpatients with DPD were prospectively evaluated. Fifty-eight outpatients with early AD were then retrospectively selected, paired in terms of age, sex, education and Mini-Mental State Examination (MMSE) score to DPD patients. In order to reduce confounding factors, early AD patients have been paired with DPD patients (2 AD for 1 DPD) as for age, sex, and MMSE score. Early AD diagnosis was made following recent criteria (7) As for DPD patients, they meet the following inclusion criteria: i) the reason of their first evaluation was cognitive complaints; ii) they did not have a diagnosis of major depressive disorder or other psychiatric illness at present and in the past; iii) they had a mild cognitive impairment (83); iv) they underwent 18F-FDG-PET within 6 months; v) they had a minimum 20 months of clinical FU. Forty-two healthy subjects were selected as controls. Exclusion criteria for all subjects included previous or current major psychiatric disorder and neurological disease. Patients with MRI evidence of major stroke or brain mass were excluded, with white matter hyperintensities, leucoaraiosis and lacunae not constituting an exclusion criterion if the Wahlund score was <3 in all regions (114). A subgroup of patients who did not undergo MRI because of claustrophobia or the presence of a metallic device were evaluated with CT. All subjects also underwent a complete neuropsychological test battery, including Mini-Mental State Examination (MMSE) (115), which was used to assess general cognition. As for patients (DPD and AD), their cognitive status (i.e., single-domain amnestic or multidomain MCI) was ascertained on the basis of their neuropsychological assessments. As stated above, major psychiatric illness including major depression was an exclusion criterion, while it was permitted a mild depressive trait ascertained by means of 15-item Geriatric Depression Scale (GDS). As for the healthy subjects, GDS score ≤5 was required for

inclusion. Morphologic brain scans for all subjects were evaluated for medial temporal lobe atrophy rate by applying the 5-point rating scale from 0 (no atrophy) to 4 (maximum atrophy) as proposed by Scheltens et al. (116). The right and left hemisphere were rated separately, the MTA score being the worst of these two values. Visual analysis was assessed by an experienced rater. Brain metabolism was evaluated by means of 18F-FDG-PET. PET Images were acquired using a Siemens Biograph 16 PET/CT scanner. Scans were acquired in 3-D mode with an acquisition time of 15 min. Images were reconstructed using an ordered subsets expectation maximization algorithm, with 16 subsets and six iterations. The CT scan was used for attenuation correction. Brain FDG PET DICOM files were exported and converted into Analyze files. The spatially normalized set of images were then FDG PET data were subjected to affine and nonlinear spatial normalization into Montreal Neurological Institute space using SPM12 (Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 7.3.0 (Mathworks, Natick,MA). The spatially normalized set of images were then smoothed with an isotropic gaussian kernel of FWHM 10mm to blur individual variations in gyral anatomy and to increase the signal-to-noise ratio. The resulting statistical parametric maps, SPM{t} were transformed into the unit of normal distribution (SPM{z}). Correction of SPM coordinates to match the Talairach coordinates was achieved by the subroutine implemented by Matthew Brett (http://www.mrc-cbu.cam.ac.uk/Imaging). Univariate analysis of variance (ANOVA) was used to evaluate the significance of differences among groups for age, education, MMSE, GDS and MTA score. Also, brain 18F-FDG-PET was compared among groups by ANOVA using SPM12 Full Factorial Analysis procedure and following all proposed steps. SPM T maps were displayed using an uncorrected $p < 0.001$ at peak level. Clusters of correlations are regarded as statistically significant if they survived the $p < 0.05$ threshold, false discovery rate corrected for multiple comparisons. Only significant clusters containing at least 50 voxels were taken into consideration. Age, education, MMSE and MTA scores were

used as nuisance variables.

Results. Demographic, neuropsychological features and MTA assessments results are listed in table 5.1. ANOVA among groups showed large areas of significant metabolic differences in posterior association cortex and medial temporal lobe (MTL), peaking at precuneus, inferior parietal lobule, middle temporal and posterior cingulate gyri (BA 7, 19, 39, 40) in both hemispheres. Significant areas were also found in left cingulate gyrus (BA 31) and caudate head and right anterior cingulate (BA 25) (Fig.5.a).

At post-hoc analysis, DPD patients compared with HC showed significant hypometabolism in the head of caudate nuclei bilaterally, and in whole left caudate nucleus (Fig.5.b). Repeating this comparison with an uncorrected $p < 0.005$ at peak level, we found a cluster extended to the left medial frontal gyrus (BA 11) (data not shown).

Compared with DPD, AD patients showed significant hypometabolism in large areas including precuneus and middle temporal gyrus (BA 7, 19, 39) in both hemispheres, left superior parietal lobule and posterior cingulate (BA 7, 30), and right cingulate gyrus (BA 31) (Fig.5.c). All data are listed in table 5.2.

Reverse comparisons (i.e. DPD vs HC and AD vs DPD) lead to not significant results (data not shown).

Discussion and conclusions. ANOVA revealed the well-known pattern of relative hypometabolism in posterior temporo-parietal and cingulate cortex in AD compared to HC or DPD. This result confirms the clinical validity of FDG-PET in the differential diagnosis of AD (26). Furthermore, the analysis revealed a relative hypometabolism in Brodmann area 25. This brain region is located in the cingulate region, in the caudal portion of the subcallosal area adjacent to the paraterminal gyrus. This is one of the least understood regions of the anterior cingulate cortex, but activity in this area is emerging as a crucial correlate of mood and affective disorder symptomatology (117). There is, indeed, an increasing evidence for a

causal role of area 25 in both the enhanced negative affect and decreased positive affect that is characteristic of affective disorders, and the cardiovascular and endocrine perturbations that accompany these mood changes. In fact, it has been demonstrated that a direct stimulation of this area produces a lowering systolic blood pressure (118) and that this is sensitive to circulating cortisol (119). Examining connection of area 25 with mood alteration, some studies found that volume of area 25 was smallest in patients with Major depression compared to healthy controls (120, 121). Other found functional abnormalities, both in increased and reduced function, in pathological mood states (119, 122). In summary, area 25 is hypothesized to be a key node in the integration of negative mood and abnormal visceral regulation: its activity has been linked with functions attributed to the default mode network, including emotion processing, attribution of affective meaning and autonomic function, as well as mentalization and autobiographical memory. Area 25 is indeed in a unique position to subconsciously link bioregulatory states with their mnemonic and emotional mood states (117). Following up on our analysis, we found a relative hypometabolism of caudate nuclei in DPD patients in comparison to healthy controls. The head of caudate nucleus is indeed involved in both memory and inhibition (123): in fact, caudate nucleus could contribute to behavior through the excitation of correct action schemas and the selection of appropriate sub-goals according to the evaluation of outcomes. These processes resulted fundamental in successful goal related action, so we can assume that caudate could be related to the cognitive processes of planning (124). The caudate may contribute to a variety of other cognitive functions as well, ranging from habit learning to attention (125). A variety of neurodegenerative disease could affect the caudate nuclei by direct neurodegeneration, deafferentation or indirect deafferentation. A typical example is Parkinson's disease, in which cognitive impairment is a common non-motor symptom related mainly, but not only, to a striato-frontal syndrome (126, 127). Patients with progression to PDD had decreased

metabolism in caudate nucleus as well as in the visual association cortex and posterior cingulate cortex during early phase of the disease, at a time when no major cognitive impairment was present yet (128). Also, in Huntington's disease some studies disclosed that rCBF in the caudate nucleus correlated with cognitive dysfunctions (129); furthermore, a relative hypometabolism of caudate has been correlated with cognitive impairment from post-traumatic brain injury (130). Aside metabolism, other studies relate morphology of caudate to cognition: indeed, hypometabolism and atrophy are consecutive stages of the same process, which is progressive in nature and is associated with the development of cognitive decline (131). Indeed, as we did not correct for brain volumetry in our study, FDG PET could have checked hypometabolism in spite of a real loss of grey matter. In effect, subcortical gray matter atrophy has been observed is not in dementia, but also in the preclinical stages of cognitive impairment (132); there is a report of a positive partial correlation between the left caudate nucleus volume and Mini-Mental State Examination (MMSE) scores, so caudate volume could be effectively correlated with cognition (133). Besides the connection between caudate and cognitive processes, there are evidences that connect this brain area with a depressive mood state. In fact, relative metabolic activity in the caudate and orbital-inferior region of the frontal lobe was significantly lower in the depressed patients with PD as compared to both nondepressed patients and control subjects (134). Then, we further explored this analysis between HC and DPD with lower statistical significance (an uncorrected $p < 0.005$) and we found an extended area from caudate nuclei to the left medial frontal gyrus (BA 11). We are aware that this are only indicative rather than statistically significant due to the liberal threshold adopted; however, it is of interest to underline that BA 11 it is also thought to play an important role in reward mediated behaviors as well as "cognitive empathy"(135).

As we stated above, there are several connections between cognition and depression

(108,110,111). This finding agrees with different influential neurobiological models of depression that directly implicate striate and anterior cingulate cortex in its etiology and/or pathogenesis, namely the limbic-cortical and the cortico-striato-pallido-thalamic model. These models give an interpretation of consequences that dysfunction within these structures has on behavior, physiology and cognition (117). In summary, both caudate and anterior cingulate cortex has been linked to the emotional tasks with cognitive demand (123-125, 136) which could be affected in depression. Our group of DPD patients, according to neurobiological correlates of their impairment, demonstrated indeed a relative hypometabolism both in caudate and BA 25. We are aware that, as the mechanism underpinning depression could have led to some compensation, reverse comparison may show some hypermetabolic areas. In fact, hyperactivity in a region consisting of limbic and paralimbic structures including area 25, was proposed to mediate the vegetative and somatic aspects of depression (137). By the way, reverse comparison between DPS vs HC did not show any significant result in our study.

Finally, comparison between DPD and AD demonstrated FDG-PET ability in the differential diagnosis between AD and DPD: AD patients showed significant hypometabolism in parietal, temporal and limbic cortical areas known to be affected in the disease. Our findings are consistent with previous findings (138), where metabolic differences in medial temporal lobe and the anterior cingulate gyrus, were not sufficient to discriminate between MCI-AD and nc-MCI patients. Thus, these areas could indeed be affected by other conditions leading to MCI, including depression (139). We are aware of some limitation of this study: first the relatively small sample, then the loss of a global atrophy rate correction, which could have led to misinterpret the relative hypometabolism findings. We are aware that depression known to be neurotoxic to medial temporal lobe structures and can contribute to their atrophy, possibly related to hypercortisolism. In conclusion, our results could pave the way

to an interesting scenario both in assessing the validity of biomarker FDG-PET either in the diagnosis of DPD, then in better understanding the mechanisms underlying this syndrome.

Paper: FDG-PET in pseudodementia **Picco A**, Arnaldi D, Pardini M, Proietti L, Grisanti S, Filippi L, Serafini G, Capitanio S, Morbelli S, Nobili F. Abstract accepted as oral communication at the 50th Annual Congress of the Società Italiana di Neurologia (SIN), Bologna 11-15 ottobre 2019 -paper in preparation

Table 5.1. Demographic, clinical and MTA scale in DPD, AD and HC.

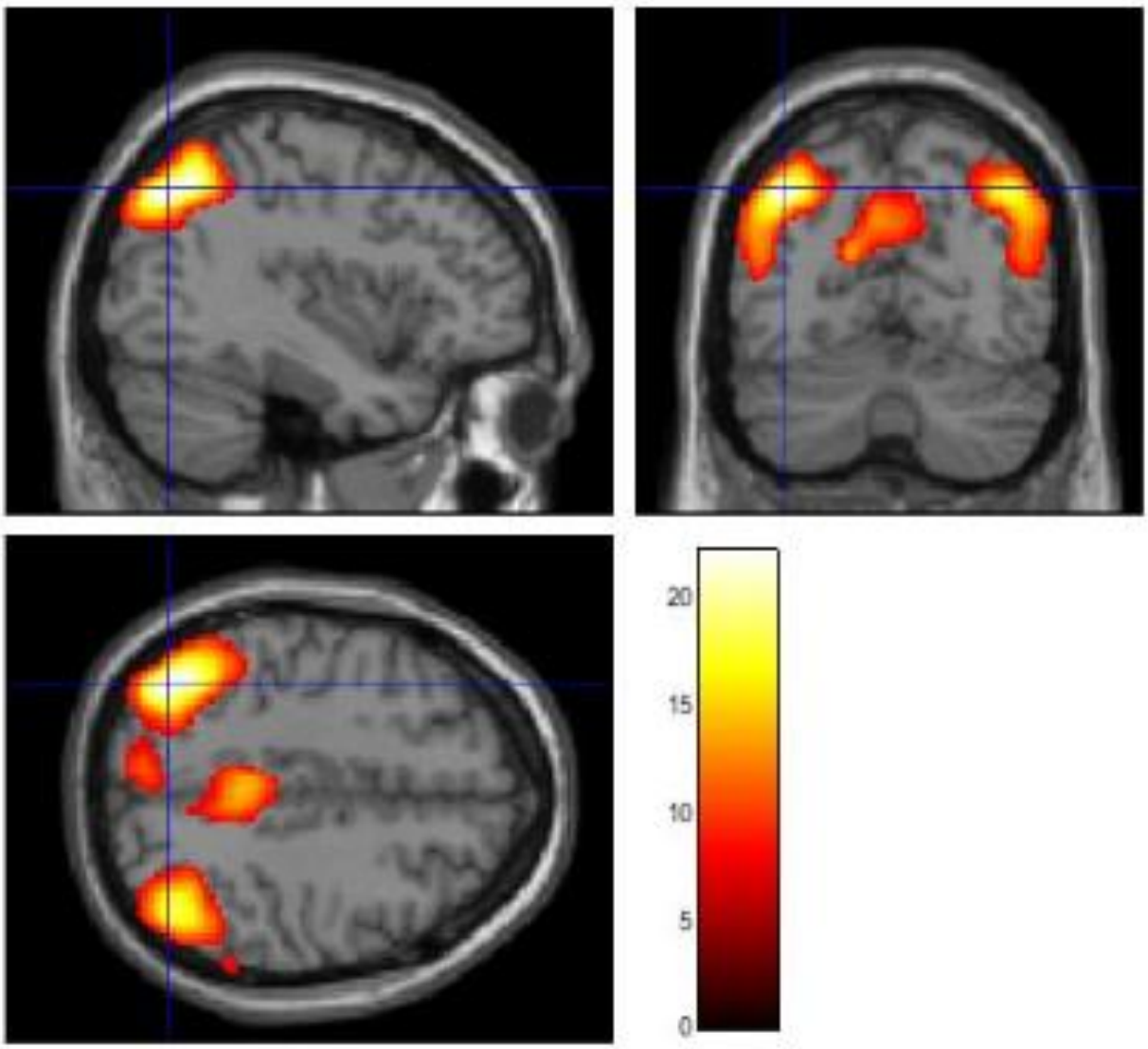
DPD: Depressive Pseudodementia patients; AD: Alzheimer's disease patients; HC: Healthy Controls; aMCI: MCI amnestic single domain; n.s.: not significant results

	DPD	AD	HC	*p-value*
Number	28	56	42	---
Gender, male %	50	32	33	---
Age, y	75.1±4.9	75.1±4.3	69.6±8.5	<0.001
Education	8.8±4.2	8.9±4.0	10.7±3.8	n.s.
MMSE score	26.1±2.8	26.2±2.7	29.2±0.8	<0.001
MCI type, aMCI %	11	46	---	---
MTA score	0.9±0.8	1.2±0.9	0.5±0.6	n.s.

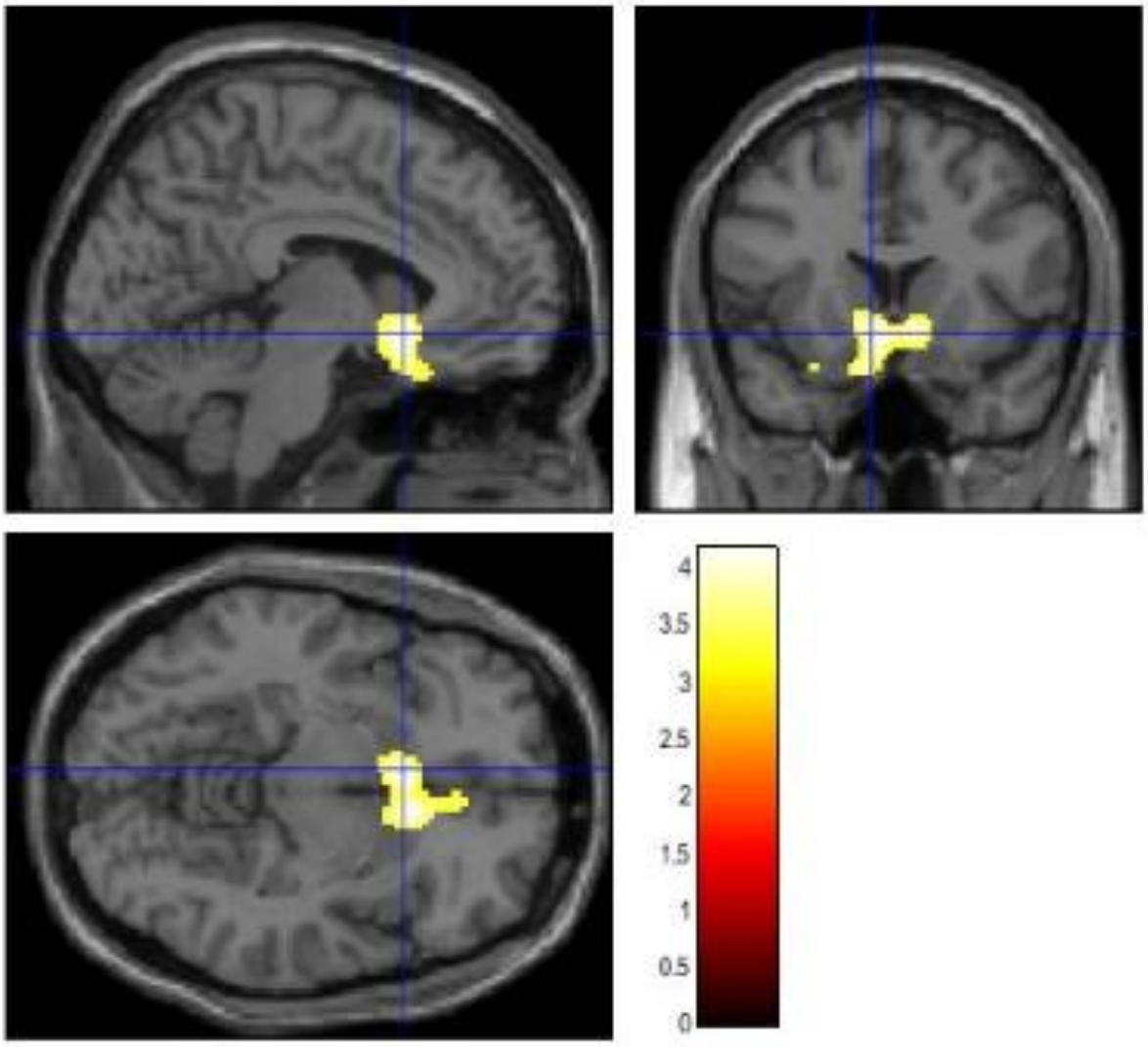
Table 5.1. PET ANOVA and post hoc analysis (uncorrected height threshold p <0.001 at the voxel level)

	Cluster level				Voxel level			
	Corrected FWE value	Corrected FDR value	Cluster extent	Cortical region	Maximum Z score	Talairach coordinates	Cortical region	Brodmann's area
ANOVA	0.00	0.00	3117	Left parietal	6.08	-37, -69, 37	Precuneus	19
				Left parietal	6.08	-39, -60, 41	Inferior parietal lobule	7
				Left temporal	5.39	-46, -62, 28	Middle Temporal Gyrus	39
	0.00	0.00	2945	Left Limbic	5.17	0.28, -44, 34	Cingulate gyrus	31
				Right Limbic	5.34	6, -56, 24	Posterior Cingulate	31
				Left Limbic	5.05	-3, -55, 24	Posterior Cingulate	31
	0.00	0.00	2489	Right Parietal	5.14	37, -67, 38	Precuneus	19
				Right Temporal	5.28	45, -61, 32	Middle Temporal Gyrus	39
				Right Parietal	3.38	56, -49, 38	Inferior Parietal Lobule	40
	0.18	0.20	530	Left Sub-lobar	4.17	-5, 14, -3	Caudate Head	
				Right Limbic	4.10	5, 18,- 3	Anterior Cingulate	25
				Right Limbic	4.01	1, 33, -2	Anterior Cingulate	25
CTR vs DPD	0.09	0.31	822	Left Sub-lobar	Caudate Gray matter	-6, 14, -2	Caudate Head	
				Right Sub-lobar	Caudate Gray matter	7, 18, -1	Caudate Head	
				Left Sub-lobar	Caudate Gray matter	-8, 13, -11	Caudate Head	
DPD vs AD	0.00	0.00	1937	Left Parietal	4.59	-35, -73, 36	Precuneus	19
				Left Temporal	4.49	-44, -62, 28	Middle Temporal Gyrus	39
				Left Parietal	4.19	-31, -66, 46	Superior Parietal Lobule	7
	0.00	0.00	1552	Right Parietal	4.42	35, -69, 40	Precuneus	19
				Right Temporal	4.35	43, -60, 25	Middle Temporal Gyrus	39
				Right Parietal	4.01	24, -57, 50	Precuneus	7
	0.01	0.01	1435	Right Limbic	4.14	2, -44, 36	Cingulate Gyrus	31
				Left Limbic	3.46	-7, -53, 8	Posterior Cingulate	30
				Right Parietal	3.30	6, -71, 36	Precuneus	7

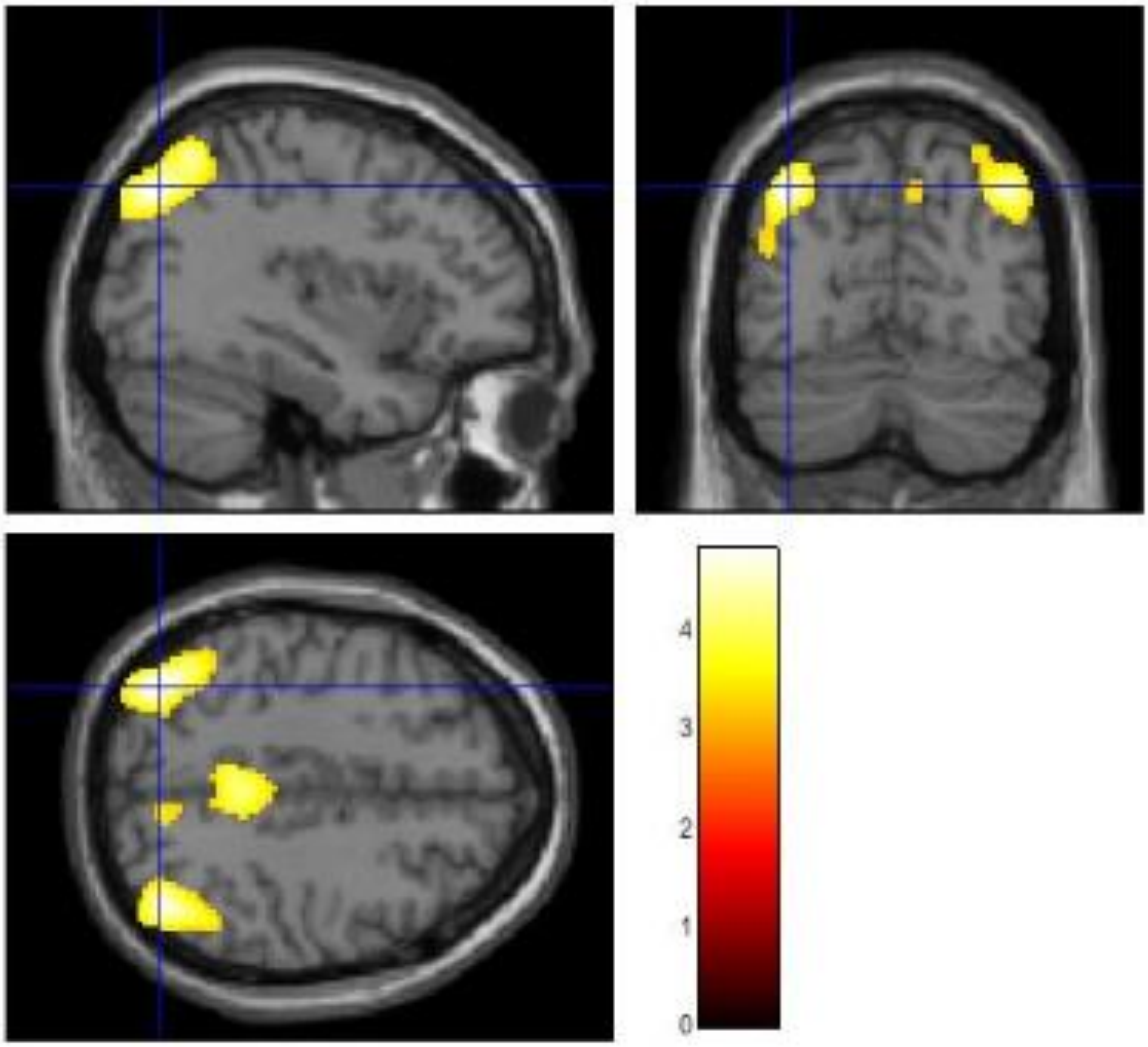
Figure 5. Results of ANOVA and post hoc comparison (SPM12; FWE corrected height threshold p<0.001 at voxel level).



5.a. ANOVA: The brain regions with a relative FDG decreased uptake reaching the statistical significance include the precuneus, inferior parietal lobule, middle temporal and posterior cingulate gyri (BA 7, 19, 39, 40) in both hemispheres, left cingulate gyrus (BA 31)and caudate head and right anterior cingulate (BA 25).



5.b. Comparison between Healthy Controls and Depressive Pseudodementia patients: The brain regions with a relative FDG decreased uptake reaching the statistical significance include the head of caudate nuclei bilaterally, and the whole left caudate nucleus.



5.c. Comparison between Depressive Pseudodementia patients and early AD patients: the brain regions with a relative FDG decreased uptake reaching the statistical significance include the precuneus and middle temporal gyrus (BA 7, 19, 39) in both hemispheres, left superior parietal lobule and posterior cingulate (BA 7, 30), and right cingulate gyrus (BA 31).

Conclusive remarks.

A great part of research in the field of AD investigates biomarkers of disease. The future of these biomarkers relies in their proper validation in all settings (analytical and clinical). In the first two papers, I cooperated with a task force of expert in AD and oncology field, who adapted a framework for BMs development used in oncology to AD. This framework included five logically sequential phases: pilot studies on analytical validity, assay development for clinical disease, prospective longitudinal repository studies, prospective diagnostic studies, and disease control studies. We assessed existing evidence based on this framework for amyloid-PET; CSF Abeta42, tau/phospho-tau; FDG-PET; hippocampal atrophy, ^{123}I-Ioflupane, ^{123}I-MIBG, and neuropsychology.

Then, I focused my PhD projects on one of the most reliable biomarkers of AD: brain metabolism by means of FDG PET and its clinical and validity among the phases of the roadmap framework. As FDG-PET rely its utility in AD not only as a diagnostic but also as a progression biomarker, we tried to use this powerful tool to clarify functional pattern able to predict future conversion to AD**.**

One of the limitations of the latter studies was to have excluded from the sample patients with other disease than AD. This was done essentially for research purpose, but hampered the possibility to explore different metabolic patterns underpinning a mild cognitive impairment not due to AD. So, we explore the utility of FDGPET in the context of a relatively frequent syndrome who could mimic early symptoms of AD, DPD. Our findings highlighted the capability of FDG PET not only to lead to a differential diagnosis, but also to improve a better understanding of the pathophysiology of DPD.

In summary, we found that:

1. By means of strategic five-phase roadmap, sufficient evidence of analytical validity (phase 1) is available for all biomarkers, but their clinical validity (phases 2 and 3) and clinical utility (phases 4 and 5) are incomplete. To complete these phases, research priorities include the standardization of the readout of these assays and thresholds for normality, the evaluation of their performance in detecting early disease, the development of diagnostic algorithms comprising combinations of biomarkers, and the development of clinical guidelines for the use of biomarkers in qualified memory clinics.
2. In the context of the five phases framework, FDG PET has fully achieved phase 1 (rational for use) and most of phase 2 (ability to discriminate AD subjects from healthy controls or other forms of dementia) aims. Phase 3 aims (early detection ability) are partly achieved. Phase 4 studies (routine use in prodromal patients) are ongoing, and only preliminary results can be extrapolated from retrospective observations. Phase 5 studies (quantify impact and costs) have not been performed. The results of this study show that specific efforts are needed to complete phase 3 evidence, in particular comparing and combining FDG PET with other biomarkers, and to properly design phase 4 prospective studies as a basis for phase 5 evaluations.
3. In order to assess the accuracy of FDG PET in discriminating MCI patients who converted to AD from those who did not, we found that MCI patients not converting to AD within a minimum follow-up time of 5 years and MCI patients converting within 5 years, baseline FDG PET and volume based analysis identified those who converted with an accuracy of 89%. However, further analysis is needed in patients with amnestic MCI who convert to a dementia other than AD.
4. With the aim to identify the cortical regions where hypometabolism can predict the speed of conversion to dementia in MCIAD we found a diagnostic-pattern, which corresponded to typical posterior hypometabolism and a conversion pattern in right middle and inferior

temporal gyri as well as in the fusiform gyrus. At Kaplan-Meier analysis, patients with hypometabolism in the prognostic pattern converted to AD-dementia significantly earlier than patients not showing significant hypometabolism in the right middle and inferior temporal cortex. The highlighted prognostic pattern is a further, independent source of heterogeneity in MCI-AD and affects a primary-endpoint on interventional clinical trials (time of conversion to dementia).

5. With the aim to explore the role of FDG PET in the diagnosis of DPD, we compared brain FDG-PET among DPD patients, patients with early AD and normal subjects. We found that DPD patients had a specific relative hypometabolism both on caudate nuclei and right anterior cingulate (BA 25). This study confirms the role of FDG PET in the diagnosis of DPD and pave the way of a better understanding of its underpinning biological alterations.

In conclusion, during my PhD I explored the necessity to define the validity of biomarkers of AD and related pathologies in patients with MCI: from operative and analytic point of view. to clinical settings. Then I focused on the real clinical implication of one of the most useful and reliable biomarkers, FDG PET. Our findings support its role of a robust progression biomarker even in a naturalistic population, and underline its importance in the early diagnosis of AD.

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1) Parkinsonism Relat Disord. 2016 Aug; 29:47-53. Functional neuroimaging and clinical features of drug naive patients with de novo Parkinson's disease and probable RBD. Arnaldi D, Morbelli S, Brugnolo A, Girtler N, **Picco A**, Ferrara M, Accardo J, Buschiazzo A, de Carli F, Pagani M, Nobili F.
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