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TESI DI LAUREA

Early phase amyloid PET versus FDG-PET in atypical dementia: the AMY-ITA multicenter study

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ANNO ACCADEMICO 2022-2023

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ABSTRACT

Background

Amyloid PET and 18F-FDG PET scans are commonly used in patients with uncertain diagnosis of Alzheimer's disease (AD). A few studies showed that early frames of amyloid PET correlate well with FDG PET images, providing perfusion-like information, thus being a potential surrogate for FDG-PET scan.

Objective

To investigate whether early florbetaben-PET (FBB-PET) imagies are comparable to those of FDG-PET in terms of regional uptake deficits in patients with a clinical suspicion of atypical AD. In addition, the change in clinical judgment by neurologists at different centers after performing FBB-PET was evaluated. Finally, through the DORIAN software, the concordance between the visual analysis of FBB- PET with a semiquantitative analysis was analyzed.

Materials and Methods

AMY-ITA is an ongoing multicenter prospective study conducted in 8 Italian centers. So far, 83 patients have been enrolled, collecting FDG-PET and FBB-PET images.

Clinical data were collected by the neurologist, who estimated the suspicion of Alzheimer's disease for all patients prior to the AMY-PET result and then confirmed or did not confirm the initial diagnosis. CSF analysis was available for 17 patients.
In visual analysis of ET images, the brain was divided into 8 different regions in both FDG and FBB-PET early-frames scans. Each region was analyzed visually and blinded in both PET scans, defining the tracer uptake abnormality, using a scale of 0 to 3.
A statistical analysis was then performed using Spearman's and Wilcoxon's tests.
DORIAN software was used, which through SUVr, ELBA, TDr and RANK methods produced a semiquantitative analysis.

Results:

The most frequent clinical variants of AD were early primary progressive aphasia, posterior cortical atrophy and young onset-AD.

Visual analysis of the brain revealed similar patterns between FBB-PET and FDGPET images of early frames; however, abnormal uptake scores were higher in FDG-PET in all regions. Spearman's test showed a statistically significant correlation in all brain regions (ρ 0.733 to 0.893, $P<0.001$). 64% of patients tested positive on AMT-PET scans and the discordance rate between the initial suspicion of amyloid positivity and the FBB-PET result was 33%. In 61% of cases the neurologist rejected the initial diagnosis and changed the clinical management. FBB-PET findings was to confirm AD in case of uninformative CSF data. A 95.5% concordance was found between visual and semiquantitative analysis of Amy-PET data.

Conclusions

Visual analysis of early-phase FBB-PET acquisitions correlated well with FDG-PET images in atypical forms of AD, offering a surrogate marker of brain metabolism. As a consequence, FBB-PET with analysis of early frames may convey added information on metabolism, reducing patient radiation exposure and health costs by avoiding FDG-PET. FBB-PET is a confirmed valid biomarker also in detecting amyloid deposition in atypical AD variants. DORIAN semiquantitative analysis confirmed visual analysis result.

RIASSUNTO

Introduzione

La PET amiloide e la PET 18F-FDG sono comunemente utilizzate nei pazienti con diagnosi incerta di malattia di Alzheimer. Alcuni studi hanno dimostrato che *early-frames* della PET amiloide correlano bene con le immagini della PET FDG, fornendo informazioni simili alla perfusione e rappresentando quindi un potenziale surrogato della FDG-PET.

Scopo dello studio

È stato studiato se le *early-frames* con florbetaben-PET (FBB-PET) sono paragonabili alla FDG-PET in termini di deficit di captazione regionale nei pazienti con un sospetto clinico di malattia di Alzheimer con presentazione atipica. Inoltre, è stata valutata la variazione del giudizio clinico da parte dei neurologi di diversi centri dopo l'esecuzione della FBB-PET. Infine, attraverso il software DORIAN, è stata indagata la concordanza tra l'analisi visiva e l'analisi semiquantitativa delle FBB-PET.

Materiali e metodi

AMY-ITA è uno studio prospettico multicentrico in corso, condotto in 8 centri italiani. Finora sono stati arruolati 83 pazienti, raccogliendo immagini FDG-PET e FBB-PET.

Nell'analisi visiva, il cervello è stato suddiviso in 8 regioni diverse sia nelle scansioni FDG che FBB-PET *early-frames*. Ciascuna regione è stata analizzata visivamente in cieco in entrambe le scansioni PET, definendo l'anomalia di captazione del tracciante, utilizzando una scala da 0 a 3.

È stata quindi eseguita un'analisi statistica utilizzando i test di Spearman e Wilcoxon. I dati clinici sono stati raccolti dal neurologo, che ha stimato il sospetto di malattia di Alzheimer per tutti i pazienti prima del risultato del FBB-PET e ha poi confermato o meno la diagnosi iniziale. L'analisi del liquor era disponibile per soli 17 pazienti.

È stato utilizzato il software DORIAN, che attraverso i metodi SUVr, ELBA, TDr e RANK ha elaborato e prodotto un'analisi semiquantitativa.

Risultati:

Le varianti cliniche più frequenti di AD sono state la malattia di Alzheimer ad *early-onset*, l'afasia progressiva primaria e l'atrofia corticale posteriore.

L'analisi visiva del cervello ha rivelato modelli simili tra le immagini FBB-PET e FDG-PET; tuttavia, i punteggi anormali assegnati erano complessivamente più elevati nella FDG-PET. Il test di Spearman ha mostrato una correlazione statisticamente significativa in tutte le regioni cerebrali (ρ 0,733-0,893, $P<0,001$). Il 64% dei pazienti è risultato positivo alla FBB-PET e il tasso di discordanza tra il sospetto iniziale di amiloide e il risultato della FBB-PET è stato del 33% dei casi. Nel 61% dei casi il neurologo ha cambiato la gestione clinica. La FBB-PET è risultata utile per confermare l'AD in caso di liquor non informativo. È stata riscontrata una concordanza del 95,5% tra l'analisi visiva e quella semiquantitativa.

Conclusioni

Le acquisizioni di FBB-PET in fase precoce sono risultate ben correlate alle scansioni FDG-PET nelle forme atipiche di demenza, offrendo un marker surrogato del metabolismo cerebrale. Di conseguenza, la FBB-PET con l'analisi degli *early-frames* può fornire ulteriori informazioni sul metabolismo, riducendo così l'esposizione alle radiazioni dei pazienti e i costi sanitari, evitando la FDG-PET. La FBB-PET si conferma un biomarcatore valido anche per rilevare la deposizione di amiloide nelle varianti atipiche di AD. L'analisi semiquantitativa di DORIAN ha confermato i risultati dell'analisi visiva.

1. ALZHEIMER'S DISEASE

1.1. Definition

Alzheimer's disease is an insidious onset and progressive neurocognitive disorder of the brain. During the last century, the definition changed simultaneously with the new diagnostic techniques and scientific discoveries introduced. Initially, Alois Alzheimer defined Alzheimer's disease as a purely pathological diagnosis, characterized by autoptic alterations. In 1984 it was introduced clinical criteria for the possible and probable presence of Alzheimer's disease; however, the definitive diagnosis was imposed on the presence of histopathological evidence: neuritic plaques and neurofibrillary tangles[1]. In 2007 the new guidelines transformed the definition of Alzheimer's disease into a clinical-biomarker construct, thanks to the introduction of MRI, cerebrospinal fluid analyses and PET that supported the clinical diagnosis[2]. In 2018 the NIA-AA proposed to define Alzheimer's disease as a biological construct, allowing to the diagnosis of this pathology using biomarkers in living people. Currently, to diagnose Alzheimer's disease one must either check for the presence of Aβ plaques and pathological tau deposits in autopsy or make an in vivo diagnosis for altered Aβ and tau biomarkers [3]. In the last fifteen years, the definition has changed from a clinical-pathological to a biological construct that allows an earlier and more sensitive diagnosis.

1.2. Epidemiology

Alzheimer's disease is the most common form of dementia. Nowadays Alzheimer's disease and other dementias are global public health problems because, as a result of the increase in life expectancy, there is an important gain for people affected by dementia, and in the next years it will continue to grow. According to World Health Organization, 50 million people have dementia, comprehending 5% of those over age 60[4]. An analysis of Alzheimer's disease during the years from 1990 to 2019

demonstrates an increase of 147% and a triplication of death[5]. In 2018 Italians with dementia were 1,279,366, and the studies advance 2.247,715 patients in 2050[6].
Two-thirds of patients are women. After a diagnosis of dementia due to Alzheimer's disease the median survival time is 6.2 years but considering patients with a mild cognitive impairment the range is from 4 to 12 years until death[7,8].

1.3. Risk factors and genetics

Risk factors are divided into non-modifiable and modifiable ones, identifying the latter allows healthcare staff to put into action a series of preventive strategies.
The modifiable risk factors for dementia are traumatic brain injury, hypertension, physical inactivity, depression, alcohol, less education, hearing loss, obesity, smoking, social isolation, air pollution, and diabetes. In particular, the first six factors are usually associated with Alzheimer's disease [9,10].
The non-modifiable risk factors are age, female gender, family history of Alzheimer's disease, and genetic predisposition. The patient's medical history should be investigated because a family history of Alzheimer's, even of the third degree, increases the risk of being affected[11]. The risk rises between 2-fold and 4-fold if a first-degree family member is affected. Despite this, there are patients with Alzheimer's without a family history[8].
To analyse genetic predisposition, it is considered separately early and late-onset Alzheimer's disease. The early-onset form is rare (less than 1% of cases), autosomal-dominant transmitted, typical of the fourth and fifth decades and it associates with mutations on chromosomes 1 (Presenilin 2), 14 (Presenilin 1) and 21 (Amyloid precursor protein)[8].
Whereas in the late-onset form, the genetic factors are more frequent but less penetrant. The strongest genetic risk factor is the APOE allele. The gene is on chromosome 19 and is involved in a major component of lipoproteins. There are three common isoforms of APOE: APOE-4 having the allele ε4 is a risk factor that determines an odd ratio of 3 in heterozygotes and 8 to 12 in homozygotes, APOE-3

with ε3 is the most common allele with a frequency of 78% in the population, APOE-2 having the allele ε2 is a protective factor.[8,12].

To date, the genetic mutations that predispose to atypical forms of dementia are mostly unknown. A minor association with APOE has been found in patients with atypical Alzheimer's disease compared to the typical form [13]. In addition, recent studies have uncovered three new loci SEMA3C, CNTNAP5 and FAM46A that may be implicated in posterior cortical atrophy[14].

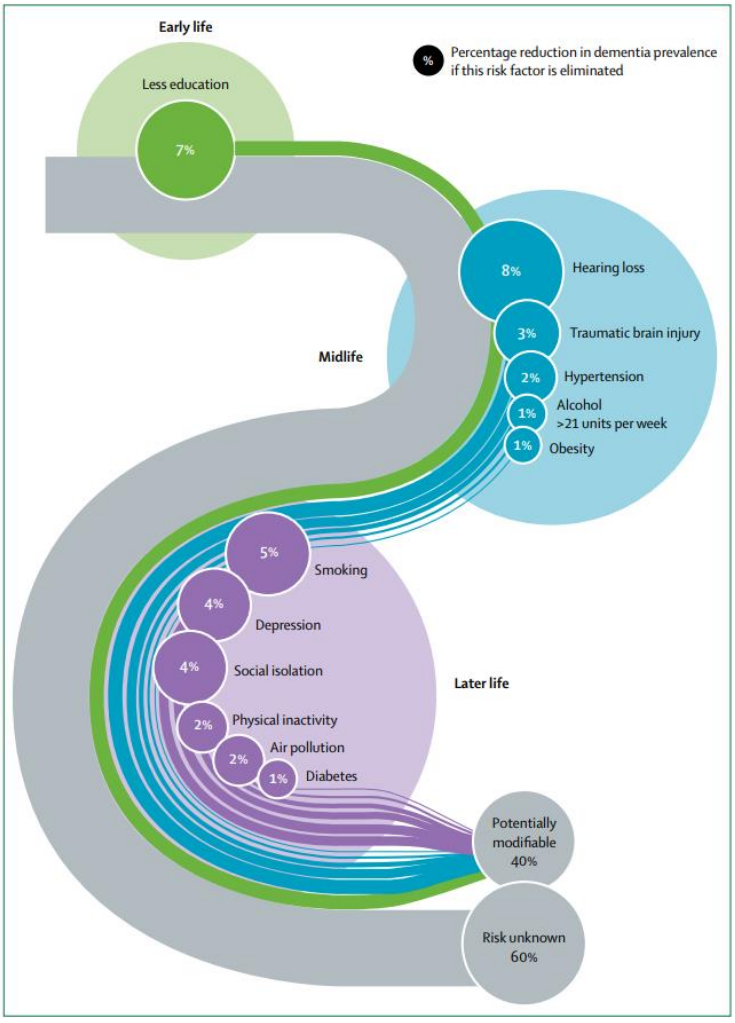

Figure 1 - Population attributable fraction of potentially modifiable risk factors for dementia [10]

1.4. Pathology

Alzheimer's disease is a neurodegenerative and prominent protein-conformational disease, due to altered formation and aggregation of soluble proteins normally produced in the brain. It was precisely because of the discovery of these amyloid plaques and neurofibrillary tangles during several autopsies in patients with decreased memory that we began to study and talk about Alzheimer's disease. Nowadays, the pathogenesis of Alzheimer's disease is still not completely known.

Physiologically amyloid precursor protein (APP) is an integral transmembrane protein with extracellular domains, whose precise function is not elucidated. APP is physiologically cleaved through the nonamyloidogenic pathway whereas, in Alzheimer's disease, cleavage occurs through the amyloidogenic pathway.

In the nonamyloidogenic pathway α-secretase claves APP and generates APPsα, a neuroprotective factor with a role in cell-substrate adhesion. Then γ-secretase clave the remaining C83 fragment and generates the soluble protein p3.

In the amyloidogenic pathway, there are two enzymatic cuts. The first cleavage, the rate-limiting step, is carried out by β-secretase called β-APP-cleaving enzyme-1 (BACE1) a membrane-spanning aspartyl protease active in the lumen. The second cleavage is performed by γ-secretase an intramembrane aspartyl protease composed of four proteins: presenilin, nicastrin, anterior pharynx-defective 1 (Aph1), and Psen2 complexed together. Respectively, the first cut, made by BACE1, leads to the formation of the soluble N-terminus of APP (sAPPβ) while the C-terminal fragment (CTF-β or C99) remains bound to the membrane. While the second cut, is carried out by γ-secretase which releases into the extracellular space insoluble and neurotoxic Aβ fragments and the APP intracellular domain (AICD) into the cytoplasm [15].

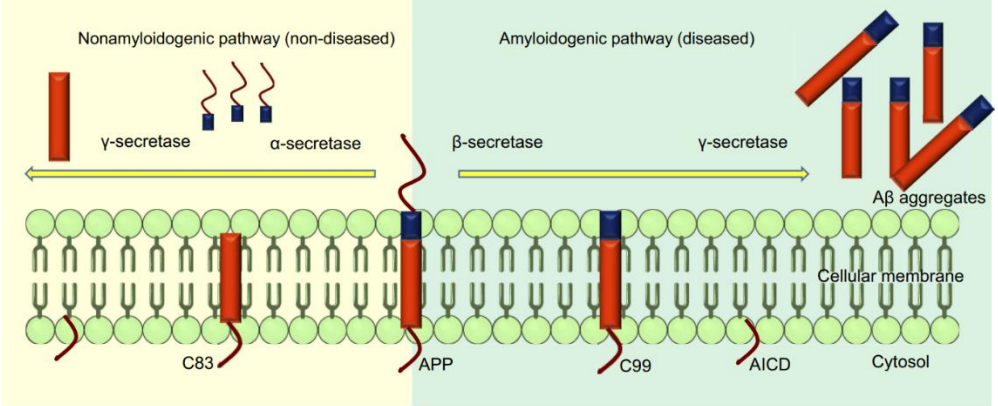

Figure 2 - Alternative splicing of APP in amyloidogenic and nonamyloidogenic pathways [15]
Abbreviations: C83, 83-amino-acid carboxyterminal; C99, 99-amino-acid membrane-bound fraction; AICD, APP intracellular domain.

In the physiological state, there are some different mechanisms implicated in the clearance of Aβ that maintain homeostasis and prevent toxic accumulation; a minimal reduction in clearance is enough to determine the accumulation of Aβ [15].

The Aβ fragments tend to organize themselves into several aggregation states including oligomers that further polymerize, forming aggregated extracellular plaques. There are two main types of Aβ polymers that have direct a role in plaque formation and induced neurotoxicity: Aβ1-40 and Aβ1-42. Aβ40 is abundant and less neurotoxic than Aβ42, which is less plentiful, highly insoluble, severely neurotoxic, and more aggregation-prone and acts as a toxic building fraction of Aβ assembly. Polymer aggregations determine the block of ion channels, alteration of calcium homeostasis, the increase mitochondrial oxidative stress, and the diminution of energy metabolism and glucose regulation, which contributes to the deterioration of neuronal health and finally to neuronal cell death[15].

Moreover, Alzheimer's disease is characterized by neurofibrillary tangles (NFTs). NFTs are paired fragments with regular helical periodicity located in intraneuronal cytoplasm and are created by hyperphosphorylation of tau protein. The protein tau is a micro-tubule-associated protein with a microtubule-binding domain, through which it binds to the microtubules, allowing them to be stable and form interconnecting bridges between contiguous microtubules.

It is currently believed that Aβ accumulation is the upstream pathophysiological event in Alzheimer's disease and that it may operate as a trigger/facilitator of

downstream molecular pathways, including hyperphosphorylation of the protein tau, and consequently tau misfolding, tau-mediated toxicity, accumulation in tangles and tau diffusion. All this leads to abnormal loss of communication between neurons and signal processing and finally apoptosis in neurons [15,16].

Finally, in reply to the aggregation of amyloid plaques and neurofibrillary tangles, there is infiltration and activation of microglia. Microglia are resident phagocytes of the brain with the role of maintaining neuronal plasticity and synapse remodeling. This activates the inflammatory response that attempts to cleanse the brain, but most often proves to be ineffective. To date, little is known about the role of microglia and neuroinflammation in Alzheimer's disease; it is an area of active research[15].

1.5. Typical clinical presentation

The most frequent presentation of Alzheimer's disease is the typical form, characterized by epiodic memory impairment, accompanied or followed by other cognitive dysfunctions such as anomia, visuospatial function, attention, and frontal/executive disturbances. Progressively, may be present also behavioral problems.

Currently, Alzheimer's disease is considered a continuous evolution from a preclinical phase marked by positivity to biomarkers, up to a phase of dementia[3,17]. The clinical stages of evolution take 15-25 years[18]. The progression is consecutive to the neurodegeneration, usually without a direct correlation between symptoms and brain atrophy because of cognitive reserve compensation.

1.5.1. Subjective cognitive decline

Subjective cognitive decline (SCD) refers to a self-experienced persistent decline in cognitive skills in patients who were previously in normal status, without comorbidities explaining this perception and not related to an acute event. This perception is reported by the subject or occasionally by close people but is not confirmed by the cognitive tests performed. Since these perceptions cannot be

objectified, such patients do not meet the criteria for mild cognitive impairment[19,20]. In some recent studies, an increased risk of developing MCI and dementia was seen in patients with SCD[20], hence the decision to assess clinical development over time by administering follow-up tests.

1.5.2. Mild Cognitive Impairment

Mild cognitive impairment (MCI) is the first clinical stage of Alzheimer's disease. According to DMV-5[21], MCI is a modest cognitive decline in one or more cognitive domains (complex attention, executive function, learning and memory, language, perceptual-motor, or social cognition) with the maintenance of daily activities. In contrast to SCD, in MCI cognitive impairment is objectifiable. The insight of disease is usually present and often leads to depression. In addition, it should be remembered that depression may be the very cause of memory problems, this should not be confused with MCI[8].

1.5.3. Major cognitive impairment

According to DSM-5[21], Major cognitive impairment or Dementia refers to a significant decline in one or more cognitive domains (complex attention, executive function, learning and memory, language, perceptual-motor, or social cognition) with independence in daily activities. The key distinction between MCI and dementia is the individual's loss of autonomy in daily life, this is measured by the ADL (activities of daily living) and IADL (instrumental activities of daily living) indices. Dementia can be subdivided into mild, moderate, and severe[8,17].

1.6. Atypical clinical presentation

Particularly in the last decade, due to the increasing use of imaging and fluid biomarkers, it is possible to make a diagnosis of Alzheimer's disease in those patients who present with atypical scenarios or have mild symptomatology. Atypical presentations are all those that do not have a main involvement, at least not at the

time of onset, of episodic memory. Often the onset of symptoms occurs in younger subjects than the typical presentation[22]. Posterior cortical atrophy, primary progressive aphasia, behavioral variant of frontotemporal dementia, corticobasal syndrome and rapid progressive dementia are considered as atypical presentations of a possible Alzheimer's disease. All of these are pathologies that are classified as stand-alone pathologies, however, in a certain percentage they can be determined by and/or associated with the amyloid accumulation and thus go to form the atypical presentation of Alzheimer's[23]. In addition, atypical forms also include the early onset of Alzheimer's.

1.6.1. Early-onset Alzheimer disease (eoAD)

Patients with early-onset Alzheimer's disease present clinical features similar to patients with a typical presentation, but the age of symptomatic onset is premature, before the age of sixty-five, and they often have a rapid progression to dementia and a shorter relative survival time [23]. The frequency of this variant amounts to 5-10% of total cases[24]. In most cases, the clinic is similar to the typical presentation with memory disorders, but in a quarter of cases, it presents with focal symptoms affecting visual-spatial, executive or language functions. 92 to 100 percent of EOAD cases are genetically determined by rare but high-penetrance autosomal dominant mutations in APP, PSEN1 and PSEN2[25].

1.6.2. Posterior cortical atrophy (PCA)

Posterior cortical atrophy is a clinical syndrome with a progressive visual or visuospatial impairment. This syndrome is among the atypical presentations of Alzheimer's as many studies have reported that in more than 75% of cases a primary or co-existing AD.; in the other cases are associated with further neurodegenerative pathology[26]. The onset is early between the fifth and sixth decade[27].

Initially, the patient reports problems with vision, difficulty in driving, reading, finding objects in front of him, and difficulties in performing activities such as dressing.

Typically, the patient presents visuospatial and perceptual deficits such as visual distortion, space alteration and disorientation, simultanagnosia, reading difficulties, dressing apraxia, prosopagnosia, and Balint syndrome (ocular motor apraxia, optic ataxia, simultanagnosia). Then presents non-visual impairments including dyspraxia, Gerstmann syndrome (acalculia, finger agnosia, agraphia, and left-right disorientation)[23,26,27]. The insight is often retained and can cause a release of anxiety[27].

Due to the heterogeneous clinical presentation, patients may be divided into three subgroups[23]:

- Biparietal (dorsal) variant: limb, constructional, and dressing apraxia, Balint syndrome, Gerstmann syndrome.
- Occipitotemporal (ventral) variant: alexia, apperceptive prosopagnosia.
- Primary visual (caudal) variant: visual field defects and diminished visual acuity, that determined a decrease of visual functions.

Patients often consult an ophthalmologist instead of a neurologist, leading to a diagnostic delay of several years[26].

1.6.3. Primary progressive aphasia (PPA)

Primary progressive aphasia is a clinical syndrome with a predominant language dysfunction with a significant impact on daily life. The onset is insidious, and the evolution is progressive and gradual. In the early stage, the predominant alteration is aphasia, the other cognitive domains are affected later [28].

PPA is split into three variants:

- Logopenic variant (lvPPA) characterizes by difficulty to find words and anomia, moderate reduction of speech rate with phonemic errors and paraphasia, and sentence repetition impaired, whereas a while comprehension

and grammar are unaffected. Several patients show up with memory impairment[23,28,29].

- Semantic variant (svPPA) characterizes by difficulty to find words and anomia, impairment in understanding words, loss of object knowledge but the ability to use it remains intact, and trouble matching a word to a picture or the colour of an object[28,29].
- Nonfluent/agrammatic variant (nfvPPA) characterizes by problems during sentence formation with reduced speed, presence of apraxia, agrammatical, and paraphasia. In addition, comprehension is consistently impaired as is repetition, which is difficult even on words only[28,29].

Different forms have a distinct prevalence of amyloid positivity: about 86% in lvPPA, 20% in nfvPPA, and 16% in svPPA[30]. The logopenic variant is the most frequently associated with the atypical presentation of Alzheimer's disease.

It should be noted that the language domain is the first to be affected by PPA, whereas the typical manifestations of Alzheimer's disease are involved in the dementia phase.

1.6.4. Behavioral variant or frontal syndrome (bvFTD or FS)

The behavioral variant of frontotemporal dementia (bvFTD) is an insidious and progressive neurodegenerative disorder defined by a decline in social cognitive functions and a modification of personality and behavior[31,32]. The insight is already scarce in the early stage.

The most prevalent features are apathy even though it is present in other dementias, then inertia, loss of empathy, disinhibition, executive function impairment, stereotypic and obsessive-compulsive behaviors, hyperorality, dietary changes, diminished personal care, speech problems, and later even memory alterations[31–33]. The clinical presentation is very variable and often correlates with the damage detected by imaging techniques[33].

Daily life in patients with bvFTD is quickly and severely affected, resulting in a lowered quality of personal, social, and family life[33].

The diagnosis is clinically suspected in those patients who present with altered behavior, driving problems such as driving on the wrong side, the presence of sexual disinhibition, or urinary incontinence that often does not cause embarrassment but rather a detachment.

The probability of patients with Alzheimer's disease experiencing a behavioral variant syndrome of frontotemporal dementia is 10-15%[34] and the probability of patients with bvFTD presenting AD is between 7 to 20%[23].

1.6.5. Corticobasal syndrome (CBS)

Corticobasal syndrome (CBS) is a clinical diagnosis due to several possible pathologies including corticobasal degeneration, Alzheimer's disease, and progressive supranuclear palsy. Corticobasal degeneration is owed to the deposit of hyperphosphorylated 4-repeat tau isoforms[8,35]. Corticobasal syndrome and corticobasal degeneration are not synonymous, not all patients with CBS have corticobasal degeneration and vice versa. Between 15% and 54% of CBS are related to Alzheimer's pathology[23].

CBS is presented with cortical and extrapyramidal signs. The cortical signs are apraxia, cortical sensory loss, alien limb phenomena, Neglect hemisensory syndrome, visuospatial deficits, focal o segmental myoclonus and Gerstmann syndrome. The extrapyramidal signs are asymmetrical Parkinsonism, rigidity, bradykinesia, dystonia and, sometimes, tremor. Language may also be affected by apraxia or non-fluent aphasia[8,23,35]. The alien limb phenomena is pathognomonic but not frequent, whereas useless limb is common[8].

1.6.6. Rapid progressive dementia (RPD)

Rapid progressive dementia (RPD) is typically considered a condition that progresses in less than one to two years, often in a few months, from the initial symptom to dementia. The prototypical RPDs are prion diseases such as Creutzfeldt-Jakob disease, however, the common causes are non-prion diseases such as vascular, toxic

metabolic, malignancy, and neurodegenerative disease. Of course, the importance is to have a correct diagnosis to start treatment. In general, RPD is rare, and the diagnosis is not immediate. The first symptoms of dementia can be different due to the etiologic cause: in neurodegenerative dementia, it depends on the affected area[36]. Nonprion neurodegenerative diseases are often the most common forms of RPD[36]. It should be borne in mind that cognitive decline in patients with Alzheimer's disease often occurs alternating between periods of rapid deterioration and others of pseudo-stability. In fact, some people may present with an undiagnosed initial mild decline that suddenly worsens in association with periods of stress such as hospitalization or infection[36].

2. DIAGNOSIS OF ALZHEIMER'S CONTINUUM

2.1. Biological diagnostic criteria for Alzheimer's Disease

As seen above, the diagnosis of Alzheimer's disease has varied over the years and is constantly evolving. The definitive diagnosis can only be made through autopsy findings of the presence of amyloid plaques and neurofibrillary tangles. Nowadays, a definitive anatomical-pathological diagnosis is no longer commonly made, but it is preferred to use ATN biological classification.

In 2018 National Institute on Aging—Alzheimer's Association (NIA-AA) proposed new diagnostic criteria through the ATN classification, based on biomarkers grouped into those of beta-amyloid deposition, pathologic tau, and neurodegeneration (ATN).[3] This classification makes it possible to diagnose Alzheimer's disease independently of clinical symptoms, thus enabling earlier diagnosis by making a pre-clinical diagnosis and also identifying patients with atypical Alzheimer's[37].

The biomarkers analysed are:

- Beta-amyloid (A)**:** the deposition of beta-42 amyloid aggregated in extracellular plaques is the primary marker of neurodegeneration due to Alzheimer's disease. Amyloid deposition may be detected in vivo through different techniques such as cerebral spinal fluid (CSF) protein analysis and positron emission tomography imaging with an amyloid-specific radiopharmaceutical tracer (Amyloid-PET).

- Phosphorylated-Tau presence (T): aggregated of phosphorylated-Tau (p-Tau) is the second core marker of amyloid pathology and is thought to lead to neurofibrillary changes. Currently, in Italy, the measurement is detected trough the cerebrospinal fluid, in other countries tau PET is also performed.

- Neurodegeneration (N): progressive cerebral atrophy is a characteristic feature of neurodegeneration. Brain imaging provides positive support for the clinical diagnosis of dementia and allows to exclude of lesions (tumors, stroke, infections, etc…) causing cognitive decline. But neurodegeneration can be due to several causes, it is an unspecific biomarker for AD. MRI, FDG PET, and the amount of total tau in the cerebrospinal fluid are used to assess the presence of neurodegeneration.

The pathophysiological evolution of Alzheimer's disease is associated with a contemporary biologic profile evolution preceding clinical manifestation. To date, the most reliable pattern of positivity of various biomarkers over time predicts the following sequence: amyloid alterations, synaptic dysfunction, neuronal damage, and finally structural brain damage (figure 3)[16,38].

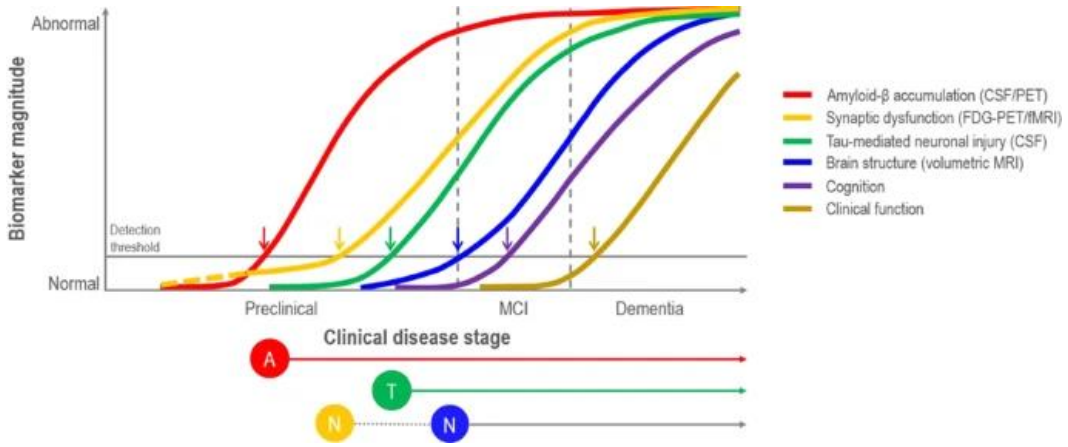

Figure 3 - Hypothetical biomarker evidence-driven model of AD [16]

Regardless of the diagnostic technique used, the three biomarkers are evaluated as positive or negative. The different combinations make it possible to identify eight different "biomarker profiles", which in turn can be grouped into three "biomarker categories" (see table I):

- Individuals with normal AD biomarkers
- Patients with amyloid beta positivity who therefore belong to the Alzheimer's continuum
- Patients with abnormal T and/or N but normal amyloid biomarker.

Alzheimer's continuum is all combinations that show amyloid positivity. If it is associated with the presence of phosphorylated tau, regardless of the presence or absence of neurodegeneration, a biological diagnosis of Alzheimer's disease can be made.

Whereas if it is associated with negativity of the T and N biomarkers we talk about Alzheimer's pathological change, i.e. an early stage of Alzheimer's disease. Finally, if A and N are positive, but T is negative, then it must be ruled out that the neuronal damage or neurodegeneration is due to other causes and not Alzheimer's disease.

Biomarker profiles and categories

AT(N) profiles	Biomarker category	
A-T-(N)-	Normal AD biomarkers	
A+T-(N)-	Alzheimer's pathologic change	Alzheimer's continuum
A+T+(N)-	Alzheimer's disease	
A+T+(N)+	Alzheimer's disease	
A+T-(N)+	Alzheimer's and concomitant suspected non Alzheimer's pathologic change	
A-T+(N)-	Non-AD pathologic change	
A-T-(N)+	Non-AD pathologic change	
A-T+(N)+	Non-AD pathologic change	

Table I - Biomarker profiles and categories. Abbreviation: AD, Alzheimer's disease. [3]

Alzheimer's clinical syndrome

The entry of biomarkers into clinical practice has changed the diagnostic process and made it possible to define Alzheimer's disease in living patients regardless of the presence of symptomatology. Obviously, this resulted in the need to introduce terminology for patients who presented with clinical symptoms suggestive of Alzheimer's disease. The term "Alzheimer's clinical syndrome" was introduced to define patients who have single- or multiple-domain memory deficits or have atypical variants suggestive of possible Alzheimer's disease[3]. The criteria required to make a diagnosis of Alzheimer's clinical syndrome are those proposed by the NIA-AA in 2011 for the previous clinical definition of Alzheimer's disease[3,37,39,40].

It is however recommended to proceed with the biological diagnosis because, as studies have shown, between 10% and 30% of patients with Alzheimer's clinical syndrome have a discrepant result at both amyloid PET and CSF and at autopsy[3,41,42].

In clinical practice in the elderly with medium to low life expectancy who present

with a clinic highly suggestive of AD, we are often limited to making a diagnosis of Alzheimer's clinical syndrome.

Preclinical Alzheimer's disease

The preclinical stage is the period prior to the onset of the first clinical symptoms in patients with biomarkers positivity or in carriers of an autosomal dominant monogenic mutation. These subgroups are referred to as "asymptomatic at risk" and "the presymptomatic", respectively[43,44]. In "asymptomatic at risk for AD" patients, amyloid positivity alone does not give evidence of a certain development into AD, whereas if it is associated with tau biomarker positivity, then, there may be a rapid development leading to the appearance of symptoms[44]. Identifying people with preclinical Alzheimer's has increasing relevance in view of new medical therapies.

2.2. Diagnostic criteria of atypical presentation of Alzheimer's disease

2.2.1 Diagnostic criteria eoAD

The diagnosis of EOAD involves the same criteria as the typical form. In addition, a genetic counseling is requested to search the blood for known mutations. The result does not change the therapeutic management but allows the aetiological cause of the disease to be defined and family members to be informed[23,24].

2.2.2. Diagnostic criteria PCA

The diagnosis of PCA is based on a three-level classification structure. Level 1 includes clinical, cognitive, neuroimaging, and exclusion criteria for other pathologies that could give rise to such symptoms secondarily. Level 2 distinguishes between the presence or absence of clinical signs for other neurodegenerative diseases in PCA-plus and PCA-pure, respectively. Level 3 determines the definite diagnosis of underlying pathology in association with PCA. In particular, the

diagnostic criteria attributable to the co-presence of AD, Lewy body disease, corticobasal degeneration, and prion disease[45].

Core features of the PCA clinico-radiological syndrome (classification level 1)

Clinical features (all three must be present): insidious onset, gradual progression and prominent early disturbance of visual ± other posterior cognitive functions.

Cognitive features:

- At least three of the following must be present as early or presenting features ± evidence of their impact on activities of daily living: space perception deficit, simultanagnosia, object perception deficit, constructional dyspraxia, environmental agnosia, oculomotor apraxia, dressing apraxia, optic ataxia, alexia, left/right disorientation, acalculia, limb apraxia (not limb-kinetic), apperceptive prosopagnosia, agraphia, homonymous visual field defect, finger agnosia.
- All of the following must be evident: relatively spared anterograde memory function, relatively spared speech and nonvisual language functions, relatively spared executive functions, relatively spared behavior and personality.

Neuroimaging: predominant occipito-parietal or occipito-temporal atrophy or hypometabolism or hypoperfusion on MRI/FDG-PET/SPECT

Exclusion criteria:

- Evidence of a brain tumor or other mass lesion sufficient to explain the symptoms
- Evidence of significant vascular disease including focal stroke sufficient to explain the symptoms
- Evidence of afferent visual cause (e.g., optic nerve, chiasm, or tract)
- Evidence of other identifiable causes for cognitive impairment (e.g., renal failure)

Classification of PCA-pure and PCA-plus (classification level 2)

- PCA-pure: individuals must fulfill the criteria for the core clinico-radiological PCA syndrome (level 1), and not fulfill core clinical criteria for any other neurodegenerative syndrome.
- PCA-plus: individuals must fulfill the criteria for the core clinico-radiological PCA syndrome (level 1) and also fulfill core clinical criteria for at least one other neurodegenerative syndrome, such as Alzheimer's disease, dementia with Lewy bodies, Corticobalsal syndrome, vascular dementia or prion disease.

Diagnostic criteria for disease-level descriptions (classification level 3)

For the diagnosis of posterior cortical atrophy associated with Alzheimer's disease, one must meet PCA syndrome criteria (classification level 1) plus in vivo evidence of Alzheimer's pathology (at least one of the following):

- Decreased Aβ1–42 together with increased T-tau and/or P-tau in CSF
- Increased tracer retention on amyloid PET
- Alzheimer's disease autosomal-dominant mutation present (in PSEN1, PSEN2, or APP)
- If autopsy confirmation of AD is available, the term definite PCA-AD would be appropriate.

2.2.3. Diagnostic criteria PPA

The diagnostic criteria for PPP include general inclusion and exclusion criteria and additional criteria specific to each of the three clinical variants. The core criteria allow differential diagnosis with other neurodegenerative diseases. The diagnostic criteria for the different variants allow a specific diagnosis and include clinical, imaging-supported and pathological criteria[28].

Inclusion and exclusion criteria for the diagnosis of PPA:

Inclusion: all three criteria must be positive:

- most prominent clinical feature is difficulty with language
- these deficits are the principal cause of impaired daily living activities
- aphasia should be the most prominent deficit at symptom onset and for the initial phases of the disease

Exclusion: all criteria must be negative:

- pattern of deficits is better accounted for by other nondegenerative nervous system or medical disorders
- cognitive disturbance is better accounted for by a psychiatric diagnosis
- prominent initial episodic memory, visual memory, and visuoperceptual impairments
- prominent, initial behavioral disturbance

Diagnostic features for the nonfluent/ agrammatic variant PPA

1. Clinical diagnosis of nonfluent/agrammatic variant PPA:
    - at least one of the following core features must be present:
        - agrammatism in language production
        - effortful, halting speech with inconsistent speech sound errors and distortions (apraxia of speech
    - At least 2 of 3 of the following other features must be present:
        - Impaired comprehension of syntactically complex sentences
        - Spared single-word comprehension
        - Spared object knowledge
2. Imaging-supported nonfluent/agrammatic variant diagnosis:
    - in addition to fulfilling clinical criteria, imaging must show one of the following results:
        - predominant left posterior fronto-insular atrophy on MRI
        - predominant left posterior fronto-insular hypoperfusion or hypometabolism on SPECT or PET

Diagnostic criteria for the semantic variant PPA

1. Clinical diagnosis of semantic variant PPA:
    - both of the following core features must be present:
        - impaired confrontation naming
        - impaired single-word comprehension
    - at least 3 of the following other diagnostic features must be present:
        - impaired object knowledge, particularly for lowfrequency or low-familiarity items
        - surface dyslexia or dysgraphia
        - Spared repetition
        - Spared speech production (grammar and motor speech)
2. Imaging-supported semantic variant PPA diagnosis:
    - in addition to fulfilling clinical criteria, imaging must show one of the following results:
        - Predominant anterior temporal lobe atrophy
        - Predominant anterior temporal hypoperfusion or hypometabolism on SPECT or PET.

Diagnostic criteria for logopenic variant PPA

1. Clinical diagnosis of logopenic variant PPA
    - both of the following core features must be present:
        - impaired single-word retrieval in spontaneous speech and naming
        - impaired repetition of sentences and phrases
    - it least 3 of the following other features must be present:
        - speech (phonologic) errors in spontaneous speech and naming
        - spared single-word comprehension and object knowledge
        - spared motor speech
        - absence of frank agrammatism
2. Imaging-supported logopenic variant diagnosis

- in addition to fulfilling clinical criteria, imaging must show one of the following results:
    - predominant left posterior perisylvian or parietal atrophy on MRI
    - redominant left posterior perisylvian or parietal hypoperfusion or hypometabolism on SPECT or PET

2.2.4. Diagnostic criteria bvFTD

The international behavioral variant frontotemporal dementia criteria allow mainly clinical diagnosis with more limited radiological and histopathological support[46].
At onset or during progression patients with bvFTD have often psychiatric symptoms, therefore a differential diagnosis must be made with psychiatric pathologies[32].

2.2.5. Diagnostic criteria CBS

The current criteria date from 2013 and are mainly based on the diagnosis of corticobasal degeneration, which may present as a corticobasal syndrome. As mentioned earlier CBS may be associated with amyloid accumulation and be included among the atypical presentations.
The diagnostic criteria are divided into probable or possible CBS. The diagnosis of probable CBS involves an asymmetric presentation of 2 of a) limb rigidity or akinesia, b) limb dystonia, c) limb myoclonus plus 2 of: d) orobuccal or limb apraxia, e) cortical sensory deficit, f) alien limb phenomena (more than simple levitation)[47].
The diagnosis of possible CBS may be symmetric: 1 of: a) limb rigidity or akinesia, b) limb dystonia, c) limb myoclonus plus 1 of: d) orobuccal or limb apraxia, e) cortical sensory deficit, f) alien limb phenomena (more than simple levitation)[47].
These criteria are insufficient to make a diagnosis of CBS associated with AD, which is why CSF analysis, neuroimaging and PET are performed in cases of suspicion[23].

2.2.6. Diagnostic criteria RPD

Currently, there are no diagnostic criteria for rapidly progressing dementia. It is a diagnosis by exclusion, with a series of examinations being carried out in parallel according to the clinical evolution of the specific patient. The possible tests range from haematochemical and urinary tests to MRI and analysis of the cephalospinal fluid. This allows the investigation of various possible infectious, toxic metabolic, systemic, vascular, and neurodegenerative causes [36].

3. DIAGNOSTIC TECHNIQUES

3.1. Cerebrospinal fluid (CSF)

Analysis of cerebrospinal fluid (CSF) allows biological diagnosis of Alzheimer's to be made according to ATN criteria. After checking for the exclusion of contraindications such as expansive processes[48], CSF is removed by lumbar puncture and is subsequently analysed for levels of amyloid and tau protein concentration.

Currently, positivity or negativity for the amyloid biomarker in CSF is based on the decrease of Aβ42 concentration[49] and on the ratio value of Aβ42 to Aβ40. Aβ42 is most correlated with plaque formation, in fact, its decrease correlates with the presence of amyloid plaques in both amyloid PET and autopsy[50,51]. Whereas Aβ40 does not undergo significant in concentration alterations in patients with Alzheimer's disease even though its concentration is normally ten times higher than Aβ42. Considering the ratio of the two different isoforms makes it possible to decrease possible errors of inter-individual variability, increasing diagnostic accuracy[50–52].

While positivity or negativity for tau biomarker is assessed by the increase in phosphorylated tau protein (p-tau). The concentration of total tau protein (t-tau) is also measured in the CSF, which is correlated with the intensity of neurodegeneration, but not specific to Alzheimer's disease. T-tau is increased in all cases of acute brain damage or in chronic neurodegenerative disorder, such as in

Creutzfeldt-Jakob disease[53]. Among the different forms of p-tau, those most specific for Alzheimer's disease neurodegeneration are p-tau181, p-tau199 and p-tau231[54]. If both p-tau and t-tau are increased, there is an increased risk of rapid progression of Alzheimer's disease[50].

Patients with atypical presentation of Alzheimer's generally have a CSF profile similar to the typical form[13]. Although some differences were identified in some studies, for example in some patients with PCA had lower levels of p-tau and t-tau, or in fvAD higher levels of p-tau and t-tau[55].

The following are normal values of the panel classically used in accordance with the medical laboratory of the University Hospital of Padua:

- t-tau >404 ng/L
- p-tau (181) >56,5 ng/L
- β-amyloid (1-42) <599 ng/L
- amyloid (1-42)/p-tau ratio <8.2
- β-amyloid (1-40) /(1-42) ratio <0.069

In MCI under the age of seventy-five, CSF examination is performed as a second-line measure in the presence of MRI or FDG-PET positivity to investigate the etiology. Between the ages of seventy-five and eighty-five, there is still no definite indication, while typically over the age of eighty-five it is not performed[56].

3.2. Structural imaging

Structural imaging is performed in most patients with suspected dementia, regardless of the underlying aetiological cause[57]. The two structural imaging techniques are computed tomography (CT) and magnetic resonance imaging (MRI). Typically, MRI is preferred because different specific sequences can be used to investigate the encephalon and it has a higher resolution than CT. In the case of claustrophobia or contraindications to MRI, the patient can still be investigated by CT[8,57].

As seen above, the diagnosis of Alzheimer's disease is made regardless of the presence or absence of neurodegeneration[3]. The use of structural imaging has two main purposes: on the one hand to rule out possible secondary causes of cognitive impairment such as hemorrhages, stroke, multiple sclerosis, neoplasms, vascular pathologies, and hydrocephalus; on the other hand, to assess the presence of areas of atrophy and possibly to quantify their degree of severity[57,58].

The current gold standard for measuring the degree of atrophy is MRI with T1-weighted volumetric sequences. Other useful sequences to increase diagnostic accuracy are T2-weighted/FLAIR often associated with small vessel ischemic disease and extensive white matter lesions, T2 * gradient echo and SWI to evaluate regional atrophy, white matter changes and for the detection of microbleeds, DWI sequences to identify recent ischemic lesions or signal changes in the neocortical and/or striatal[12,59]. Contrast medium is not required[22].
In clinical practice it is also useful to grade neurodegeneration through atrophy scores such as hippocampal atrophy (MTA = Medial Temporal lobe Atrophy), global atrophy (GCA = Global Cortical Atrophy), chronic vascular damage (Fazekas scale) and parietal atrophy (Koedam scale).

In the different clinical presentations of Alzheimer's disease, different patterns of atrophy are associated on MRI.
In patients with typical Alzheimer's disease, the first areas affected by atrophy are the hippocampus and entorhinal cortex; these changes correlate with initial symptomatology. Deep atrophy of the posterior cingulate gyrus and adjacent precuneus is also observed[60]. Neuronal loss progresses with time and it will affect not only the entire medial temporal lobes but also the parietal and frontal cortex usually in a symmetrical fashion [12,61].

The areas most suggestive of early-onset Alzheimer’s disease are the parietal lobe, particularly the parietal associative cortex, and the temporal lobe [23,62]. Although, compared with late-onset, it is more common to find cortical atrophy than temporal-

medial atrophy[12]. In addition, the frontal lobe is more frequently involved than in the late onset. Finally, the hippocampus is less involved[23].

In PCA there is posteriorly predominant atrophy, which affects some areas over others depending on the clinical subgroup, for example, if the patient has Balint syndrome then the superior parietal lobe will also be involved[23].

In lvPPA the MRI typically shows volume loss in the left temporoparietal junction and functionally associated areas. Subsequently, atrophy expands to the anterior and medial temporal lobe, inferior parietal lobe and contralateral hemisphere[22,59].

In bvFTP the frontotemporal structures are more typically affected by atrophy, this may be even minimal in some patients and there is a risk of underdiagnosing the disease radiologically. In addition, MRI is useful in making differential diagnoses with psychiatric disorders[59].

In CBS atrophy is asymmetric and typically localized in the parietal lobe and the posterior frontal area.

3.3. Functional Imaging

As mentioned earlier, structural imaging makes it possible to highlight after a period of time the atrophic brain areas resulting from the neurodegenerative process initiated by amyloid deposition. In contrast, functional neuroimaging takes advantage of various radioactive tracers injected intravenously that through ion release allow the acquisition of images to investigate biochemical processes “in vivo” for brain metabolism, neurotransmission, or deposition systems of abnormal proteins.
The techniques used to assess the presence of neurodegeneration are SPECT and PET. In the diagnosis of Alzheimer's disease, FDG-PET is a level two test, while amyloid PET is level three. Whereas brain SPECT with the perfusion tracer 99Tc

HMPAO has a lower sensitivity than FDG-PET. To date it is only used to study cerebral flow.

3.3.1. DaTScan

It is important to perform a proper differential diagnosis between the different causes of cognitive impairment, in particular, if this involves a change in therapeutic management. Dementia with Lewy bodies typically presents a suggestive clinic with visual hallucinations, rapid eye movements, sleep disturbance, and extrapyramidal changes. If the clinical presentation is not diagnostic a DaTScan can be performed. DaTScan is a single photon emission computed tomography (SPECT) scan that uses ligands of the pre-synaptic dopamine transporter (DAT). Reduced binding is related to the loss of presynaptic dopamine. Although it is little used this technique allows for increased diagnostic sensitivity and specificity and consequently decreases the risk of misdiagnosis[63].

3.3.2. 18F-FDG PET

FDG-PET is a technique for analysing cellular metabolic capacity by detecting positrons emitted by the previously inoculated tracer. 18F-FDG is a radioactive glucose analogue in which a fluorine atom replaces the hydroxyl group (-OH) in position 2. The tracer enters the cell via the GLUT transporters, where it is phosphorylated by the enzyme 18F hexokinase-FDG-6-PO4; this form remains locked inside the cell allowing images to be acquired. Based on the amount of signal picked up by the PET scan, the level of metabolic activity present in each brain area can be determined.

In patients with cognitive impairment, the level of metabolic activity of neurons is an earlier marker of neurodegeneration than the presence of atrophy on MRI[64]. Depending on which areas are hypometabolic on FDG-PET, different more or less specific patterns, some even pathognomonic, can be identified for different underlying pathologies[64,65]. In clinical practice, FDG-PET is a second-level test that implements diagnostic accuracy by providing additional information about the

neurodegenerative pattern present and possibly the degree of severity [65]. In addition, hybrid PET/CT or PET/RM instruments are currently being used, which allow better anatomical precision to the functional image, enabling the radiopharmaceutical distribution to be correctly localized.

In patients with Alzheimer's disease hypometabolism is found mainly in parietotemporal regions including the precuneus and posterior cingulate complex[64,66]. Even with equal symptom severity, hypometabolism in early-onset AD is more prevalent and severe than in patients with late-onset AD[22]. As in structural imaging, atypical presentations of Alzheimer's disease have different areas of hypometabolism than the typical presentation: lvPPA has parietal or predominant left posterior perisylvian hypometabolism[28]; bvFTD has hypometabolism in the frontal and anterior temporal areas[23]; in CBS there is often asymmetric posterior hypometabolism or posterior cingular region, lateral temporal and medial and lateral parietal[23].

3.3.3. Amyloid PET

Amyloid-PET allows the detection in vivo of brain amyloid deposition using a radioactive tracer with high sensitivity and specificity [64,67]. Initially, the tracer used was the ^{11}C-labelled Pittsburgh compound B (PiB), but it had a half-life of only twenty minutes, making widespread use impossible. Three new ^{18}F-labelled tracers with high binding affinity for amyloid plaques and longer half-lives were approved by FDA and by the European and Italian authorities between 2012 and 2014: Florbetapir (Amyvid®, Eli Lilly), Florbetaben (Neuraceq®, Piramal) and Flutemetamol (Vizamyl®, GE Healthcare)[67]. All three are commonly used in clinical practice: in recent years, the use of this diagnostic technique as a third-level test has been consolidated.

The tracer is injected intravenously, arriving at the brain level with high initial uptake followed by wash-out of unbound tracer from cortical areas lacking fibrillar amyloid.

Images are acquired late at different times depending on the tracer used, for example, if Florbetaben is used, images are acquired 90 minutes after injection [67,68].

The scans are read by two nuclear physicians who refer to the amyloid-PET as negative or positive. In the first case the amyloid plaque deposits are absent or minimal, in the second case the plaques are moderate or high. Put simply, amyloid PET is positive if there is no differentiation between grey and white matter or if there are areas of grey matter that show uptake of the tracer. Reading these images is not easy and requires experienced medical personnel [69].

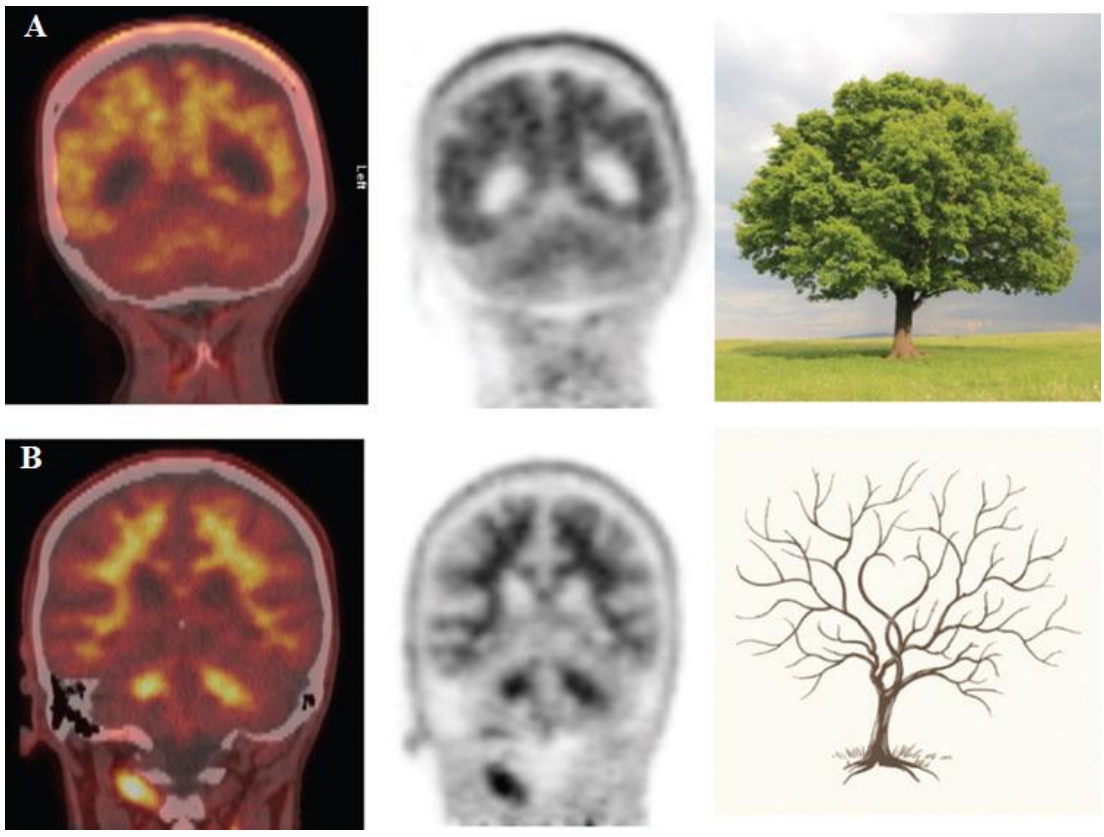

Figure 4 - Amyloid PET interpretation: (A) 'Tree-in-bloom' sign with loss of cerebral grey–white matter differentiation indicating a positive scan. (B) 'Branching tree' sign with good grey–white matter differentiation indicating a negative scan.

Amyloid-PET has been shown to be useful for the diagnosis of atypical Alzheimer's variants [64]. The amyloid-PET positive test alone does not allow for a diagnosis to be made because 20-40% of healthy people over 60 years present high levels of cerebral Aβ deposits [69]. Several autopsy studies confirm that the sites detected by amyloid-PET correspond to amyloid plaque deposits.[65]

Amyloid-PET indication according to Italian Workgroup[70]:

- patients with persistent or progressive MCI, present for at least 6 months, which remains unconclusive after functional or morphological imaging and CSF examination
- patients who meet the diagnostic criteria of possible Alzheimer's disease (NIAA criteria 2011) but with atypical clinical presentation, atypical progression, or important confounding comorbidities, with a final diagnosis that remains uncertain after functional or morphological imaging
- early onset cognitive impairment (<65 years) when the diagnosis remains questionable after functional or morphological imaging
- in case of focal cognitive syndromes when the diagnosis remains doubtful after functional or morphological imaging (progressive aphasia, progressive agnosia, progressive apraxia, cortico-basal syndrome).

On the other hand, there is no indication of the execution of Amy-PET with tracers in the following cases:

- patients who meet the criteria for probable AD, with a typical age of onset (> 75 years), probable DLB, probable PDD, amyloid angiopathy (as the positivity of the amyloid PET is not discriminatory in determining the severity or progression of the cognitive disorder)
- in asymptomatic patients even if there is a familiarity or ε4 alleles of ApoE
- patients with subjective cognitive impairment not confirmed by neuropsychological tests
- as an alternative to genotyping in subjects carrying an autosomal dominant mutation causing AD
- for non-medical purposes

Recent work, such as that involving the use of the 18F-Florbetaben tracer, has shown that the early phase of uptake in amyloid-PET is qualitatively and quantitatively superimposable to 18F-FDG-PET. This would make it possible, through amyloid-PET alone, to acquire information on cell metabolism as well [71].

4. AMY-ITA MULTICENTER STUDY

AMY-ITA is a longitudinal, prospective three-year longitudinal multicenter study involving eight Italian memory clinics: Genoa, Parma, Pavia, Prato, Perugia, Prato, Trieste and Padua. We here report preliminary data.

4.1. AIM OF AMY-ITA STUDY

Primary endpoint

To assess whether Amy-PET (by 18F-Florbetaben - FBB) using early frames is comparable to PET with 18F-FDG in detecting and graduating neurodegeneration in patients with atypical presentations (phenotypes and/or disease course) with high probability of Alzheimer's disease.

Secondary endpoints

a) To evaluate the clinician's diagnostic confidence change after 18F-FDG and after 18F-FBB (early + late)

b) To analyse the concordance and the discordance of CSF biomarkers with AMY-PET results

c) Comparison of late visual and semi-quantitative analysis with DORIAN of the early phase (0-15 min) and late phase of 18F-FBB PET

d) Correlation between different acquisition times (0-15min; 0-10min; 0-5min) of the early phase of 18F-FBB PET

4.2. MATERIALS AND METHODS

AMY-ITA is a multicenter three-year longitudinal study currently in progress with the purpose to confirm the hypothesis that early phase of Amy-PET with 18F-Florbateben is a valid surrogate marker of synaptic dysfunction like 18F-FDG. We report preliminary data from the first three years of the study.

4.2.1. Patients

From September 2021 to May 2023 83 patients were enrolled and performed 18F-FDG and 18F-FBB PET scans three months apart.

The inclusion criteria were the following:

- males and females aged less than or equal to 75 years
- clinical diagnosis of cognitive decline with atypical presentation and/or atypical course (focal variants and/or presenile onset/rapid progression)
- allowed to undergo magnetic resonance imaging at 3T with at least one 3D T1 isotropic sequence at 1 mm for no more than 3 months apart from the first scan
- available PET scan with 18F-FDG underwent for assessment of cognitive decline in last 3 months
- able to tolerate the protocol (an amyloid PET study requires a patient to be able to endure two non-consecutive brain scans of 20 minutes each)
- able to participate in follow-up clinical visits
- able to understand the informed consent and the related forms attached to the research project

The exclusion criteria were the following:

- state of pregnancy
- presence of medical devices not suitable for the study of magnetic resonance
- subjects who had previously performed a PET with tracer for amyloid
- subjects with an alternative diagnosis that could explain the cognitive decline (eg severe acute vascular lesions to the MR)
- subjects subjected to experimental drugs in the previous two years
- subjects undergoing experimental or non-experimental radiopharmaceutical procedure within ten half-lives
- any possible comorbidity that might make the procedure difficult for the patient to tolerate

The presence of a control group was not necessary since the study is aimed at investigating the performance of PET with 18F-FBB (early + late phases) in the same subjects compared to PET with 18F-FDG.

4.2.2. Study protocol

The AMY-ITA multicenter study, still in progress, has so far been conducted in eight Italian centers where PET investigations with 18F-FBB (PET/ CT or PET / MRI) have been carried out for routine clinical purposes, upon specialist request.

The protocol includes 3 visits:

Visit 1

The visit is carried out by the neurologist, during which the subjects to be enrolled are selected according to the following criteria:

- there is a clinical need to perform Amy-PET scan following standard recommendations and guidelines
- the patient meets the study inclusion criteria
- the patient, after having received a detailed presentation of the research project, expresses interest and intention to participate in it.

After obtaining the patient's informed consent, the specialist collects the clinical, diagnostic and laboratory data required for the study. The following data are required:

- 3D isotropic MR and 18F-FDG PET images, both performed no later than the previous 3 months. Diagnostic images are archived on DVD in DICOM format.
- results of any neuropsychological tests and biomarkers

The neurologist fills out a form containing all the information required by the protocol for the first neurological examination. The information required is as follows:

- subjective symptomatology at onset (memory, language, visual-spatial, apraxia, attention, behavior and executive)
- presence/absence of MCI: if present, specify the type of deficit (amnestic, non-amnestic, single domain or multiple domain) and whether it is progressing
- crude value of MMSE and/or MOCA
- possible comorbidities
- intake or not of psychoactive drugs
- dates and reports of the examinations listed above (MRI, 18F-FDG PET) and possibly DatScan, CSF and genetic tests.
- pre-test estimation of the percentage probability of the presence and absence of cerebral amyloidosis
- indicate pre-test diagnostic suspicion based on symptomatologic features and course, choosing between PCA, PPA, CBS, FS, early-onset AD (eoAD), RPD and atypical dementia for indeterminate cognitive decline (AD).

Visit 2

Performed by the nuclear medicine specialist on the day in which the patient underwent PET scan with 18F-FBB (according to the indications given below).
The following data were recorded in a specific form:

- injected activity (in MBq obtained from the syringe activity - residual activity after tracer injection)
- any suspected or established extravasation of the tracer, any movement of the patient during acquisition, injection method.

The acquired images were instead archived on DVD in DICOM format.

Visit 3

This examination is performed by the neurologist after viewing the 18F-FBB PET report and within 12 months from visit 1. The clinician indicates on the appropriate form the percentage of presence and/or absence of post-test amyloidopathy,

indicating a 'final' clinical-diagnostic diagnosis and any change in clinical judgment and management of the patient.

4.2.3. PET with 18F-Florbetaben

Each patient enrolled in the multicenter study was subjected to a PET scan with 18F-FBB according to the precise technical instructions provided by the coordinating center (UOC Nuclear Medicine of the University Hospital of Padua) in order to standardize the data obtained in different centers.

Regarding the PET acquisition:

- start the scan (in list mode) before the injection of the tracer, with the patient correctly positioned for brain study and venous access already available
- after a few seconds, proceed with an intravenous injection of 300 MBq of 18F-Florbetaben, continuing the PET acquisition for the next 15 minutes (0-15 min. Or "Early frame")
- at this stage, also acquire CT with "ultra-low dose" parameters aimed only at correcting the attenuation and not for diagnostic purposes.
- acquisition pause (the patient returns to the waiting room)
- after about 90 minutes from the injection of the radiopharmaceutical, reposition the patient and perform the late acquisition lasting 20 minutes (90-110 min. Or "late frame")
- At this stage, acquire CT with the usual parameters for a brain study

Regarding PET reconstruction:

- Matrix: 256 × 256 matrix (voxel size 2.32 × 2.32 × 2.03 mm)
- Reconstruction: 3D ordered subsets expectation maximization algorithm
- Iterations: 8 iterations
- Subset: 21 subsets
- Filter: 3-mm Gaussian

- Corrections: Standard corrections for decay, scatter, dead time, and attenuation.

Reconstruct the "Early Frame" PET 0-15 min with the above parameters and with the following times:

- 0-5 min (name the file PET_0_5)
- 0-10 min (name the file PET_0_10)
- 0-15 min (name the file PET_0_15)

Reconstruct the "Late Frame" PET 90-110 min with the above parameters and with the following times:

- 90-100 min (name the file PET_90_100)
- 90-110 min (name the file PET_90_110)

4.2.4. Data collection

The processing of personal data was carried out in accordance with current legislation (Code regarding the protection of personal data, Legislative Decree 30 June No. 196/2003 and EU regulation 2016/679). All the data necessary for the research project (visits and images), were anonymized at the satellite Center and subsequently uploaded to an electronic database accessible only to investigators via login and password; each patient was assigned a unique identifier (ID). The PET images obtained were transmitted to the coordinating Center. 15 minutes 18F-Florbetaben scan early phase and 18F-FDG scan were subjected to independent and casual visual assessment by two operators. Eight brain regions were considered for visual assessment: frontal lobe right and left, temporal lobe right and left, parietal lobe right and left, occipital lobe right and left. Signal alteration for each region was estimated by grading scale from 0 to 3 (0-irrelevant, 1-mild, 2-moderate, 3-severe). For each patient MRI T1 sequences was evaluated to identify atrophy and 90-110 minutes late-phase 18F-Florbetaben to detect amyloid deposition.

4.3. STATISTICS

Descriptive statistics were assessed to analyse the characteristics of the population, to estimate prevalence of dementia clinical syndromes considered in this study, to evaluate the prevalence of amyloid-related pathology detected by Amy-PET and CSF when available, and its contribution to clinical judgment. This analysis was assessed for the total population and for each Center. We calculate Spearman's rank correlation coefficient to evaluate the correlation of neurodegeneration scores obtained independently by visual assessment of 18-FDG-PET scan and 18F-Florbetaben scan. Wilcoxon signed-rank test was assessed to verify the hypothesis that the two neurodegeneration scores referred to the same population.

DORIAN is a research software from a INFN spin-off developed in collaboration with the University of Padua for image analysis with three pipelines: DatScan, FDG-PET and amyloid PET, the latter being the one used in this study. For the analysis of the Amy-PET images, the system requires three types of images:

- late Amy-PET: frames from 90-110 minutes post-injection were used.
- early Amy-PET: dynamic acquisition using list-mode was used and then the images were reconstructed using 3 different timeframes; 0-5 minutes, 0-10 minutes and 0-15 minutes post-injection.
- MRI: Isovolumetric MRI images were required.

Dorian analyses the images using three different methods:

- SUVr (standard uptake value ratio) calibrates the ratio of PET counts, using the number of photons detected between target regions of interest (ROIs) and a reference region, which in this case is the cerebellum. To do this, the images are first processed and calibrated [72,73].
- ELBA captures the intensity distribution patterns of the images of the entire brain. These patterns have been shown to be characteristic in PET-positive and PET-negative patients. Compared to SUVr it requires minimal image reprocessing and is not based on using ROIs[74].

- TDr (time delayed ratio) is the ratio of the mean intensities in the early phase uptake ROIs to the late phase reference ROIs. It exploits both phases to adapt both target and ROIs to each patient[75,76].
- RANK parameter considers SUVr, ELBA and TDr together giving a general value of positivity (RANK greater than 50%) or negativity (RANK less than 50%) of the Amy-PET.

4.4. RESULTS

4.4.1. Study population

To date, the multicenter AMY-ITA study has enrolled 83 patients, of whom four patients have not yet made visit 3. The study population, 48 women (58%) and 35 men (42%), had an age range from 46 to 78 years with a mean age of 66 ± 7 years. All patients were assessed for clinical decline at visit 1 with in-house neuropsychological protocols and Mini Mental State Examination (MMSE). According to DSM-5 the population consists of 54 patients with a diagnosis of minor neurocognitive disorder (65%) and 29 patients with major neurocognitive disorder (35%). The mean MMSE is 22.0 ± 5.7, range from 8 and 30. Based on the initial clinical suspicion established by the neurologists at visit 1, the diagnostic syndromes were distributed as following: 24 PPA (28,9%), 15 PCA (18,1%), 12 FS (14,5%), 9 eoAD (10,8%), 9 RPD (10,8%), 9 DA (10,8%) and 5 CBS (6,0%). See figure 5.

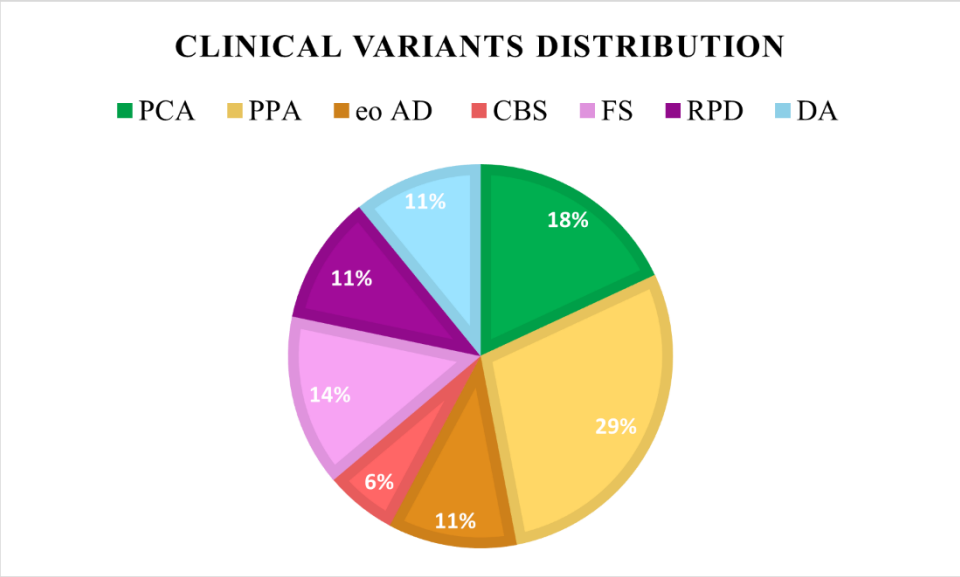

Figure 5 - Clinical variants distribution

PCA (posterior cortical atrophy), PPA (primary progressive aphasia), eoAD (early-onset Alzheimer's disease), CBS (corticobasal syndrome), FS (frontal syndrome) and RPD (rapid progressive dementia) and DA (atypical dementia)

4.4.2. Early phase 18F-Florebetaben versus 18-FDG PET

Visual assessment of both 18F-FDG PET and early frames Amy-PET in the whole study group revealed a similar spatial pattern of uptake deficits. The median values of the visual rating scale was comparable between FDG-PET and Amy-PET in each lobes. Table II reported the median values of the visual rating scale for each lobe in each hemisphere for the two PET scans.

median neurodegeneration score		
lobes	18F-FDG-PET	Amy-PET
RFL	0	0
LFL	0	0
RPL	1	1
LPL	1	1
RTL	1	1
LTL	1	1
ROL	0	0
LOL	0	0

Table II - Median neurodegeneration score. RFL (right frontal lobe), LFL (left frontal lobe), RPL (right parietal lobe), LPL (left parietal lobe), RTL (right temporal lobe), LTL (left temporal lobe), ROL (right occipital lobe), LOL (left occipital lobe).

A significant correlation between the individual scores obtained with two PET scans was obtained by Spearman's rank correlation coefficient (rS or ρ) for all lobes: RFL rS 0.836; LFL rS 0.870; RPL rS 0.893; LPL rS 0.894; RTL rS 0.852; LTL rS 0.820; ROL rS 0.738; LOL rS 0.733, all with p<0,001. See figures 6 and 7.

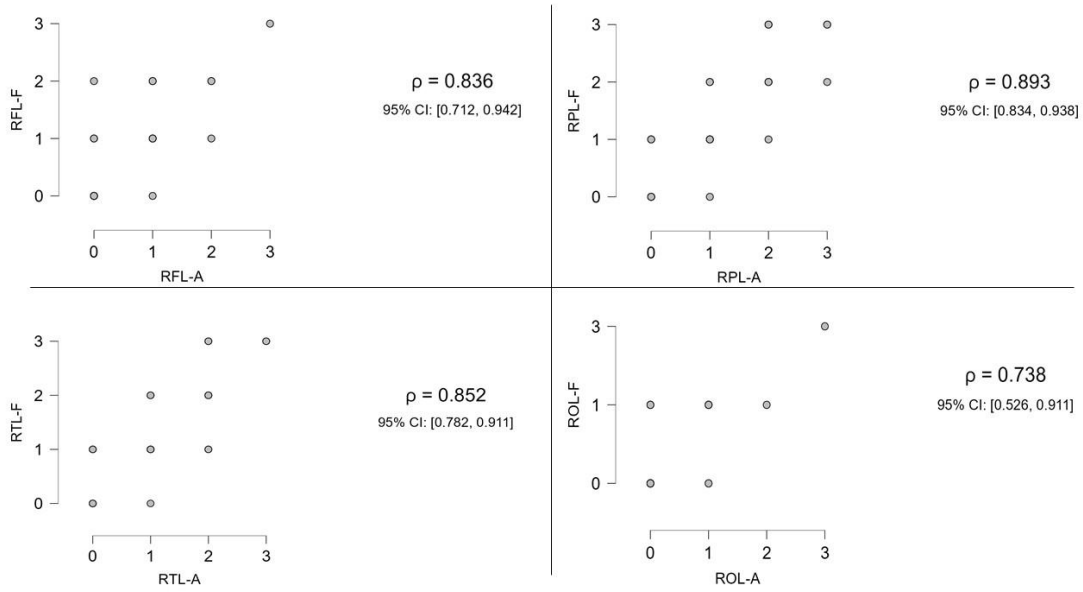

Figure 6 – Spearman correlation of visual assessment between FDG-PET and Amy-PET of right hemisphere.p<0.001. ρ (Spearman's rank correlation coefficient), RFL-F (right frontal lobe- FDG PET), RFL-A(right frontal lobe- Amy PET), RPL-F (right parietal lobe- FDG PET), RPL-A(right parietal lobe- Amy PET), RTL-F (right temporal lobe- FDG PET), RTL-A (right temporal lobe- Amy PET), ROL-F (right occipital lobe-FDG PET), ROL-A(right occipital lobe- Amy PET).

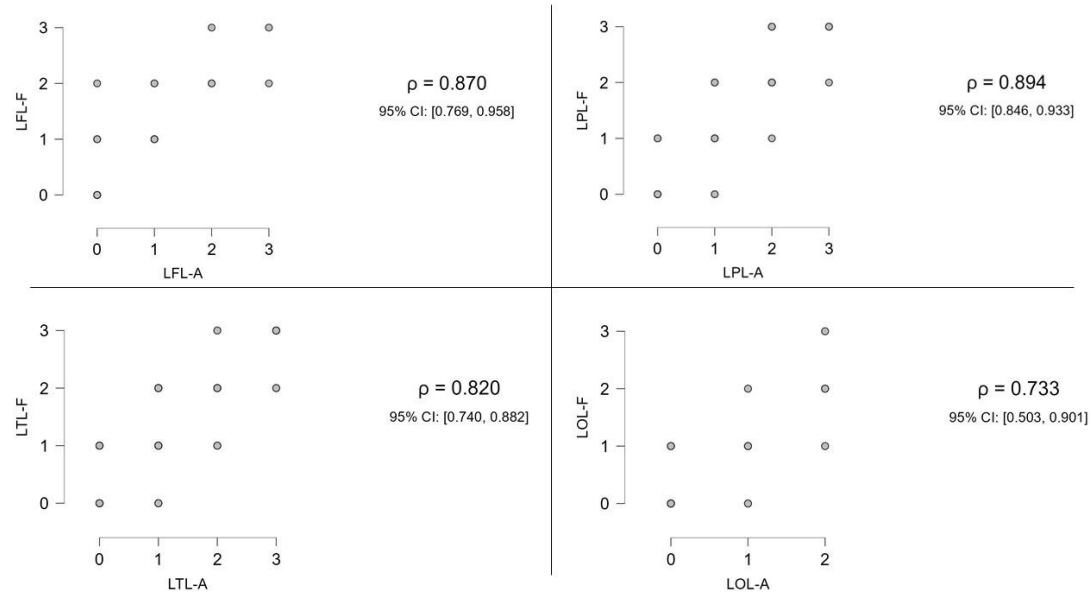

Figure 7- Spearman correlation of visual assessment between FDG-PET and Amy-PET of left hemisphere.p<0,001. ρ (Spearman's rank correlation coefficient), LFL-F (left frontal lobe-FDG PET), LFL-A (left frontal lobe-Amy PET), LPL-F (left parietal lobe-FDG PET), LPL-A(left parietal lobe-Amy PET), LTL-F (left temporal lobe-FDG PET), LTL-A (left temporal lobe-Amy PET), LOL-F (left occipital lobe-FDG PET), LOL-A (left occipital lobe-Amy PET).

The Wilcoxon test confirmed the hypothesis that the two scores are related to the same population for occipital lobes, and refused the hypothesis for frontal, parietal and temporal lobes finding a significative difference ($p<0.05$). (Table III)

Wilcoxon test			
areas	p-values	areas	p-values
RFL-F vs RFL-A	0,037	LFL-F vs LFL-A	0,009
RPL-F vs RPL-A	<0,001	LPL-F vs LPL-A	0,001
RTL-F vs RTL-A	0,001	LTL-F vs LTL-A	0,007
ROL-F vs RFL-A	0,227	LOL-F vs LFL-A	0,777

Table III - Results of the Wilcoxon test between FDG-PET and Amy-PET. P value is significative if <0,05.

4.4.3. Change of clinical's diagnostic confidence after 18F-FDG and after 18F-FBB (early + late)

Late Amy-PET was positive in 53 patients (63.9%) with a different distribution according to both the clinical syndrome suspected by the neurologist and the center of origin. For the distribution in different centers see table V. A positive late Amy-PET was more frequent in eoAD (88.9%), PPA (79.2%), RPD (77.8%) and PCA (73.3%); whereas it was less frequent in FS (41.7%) CBS (40.0%) and DA (11.1%) (Figure 8). See table V.

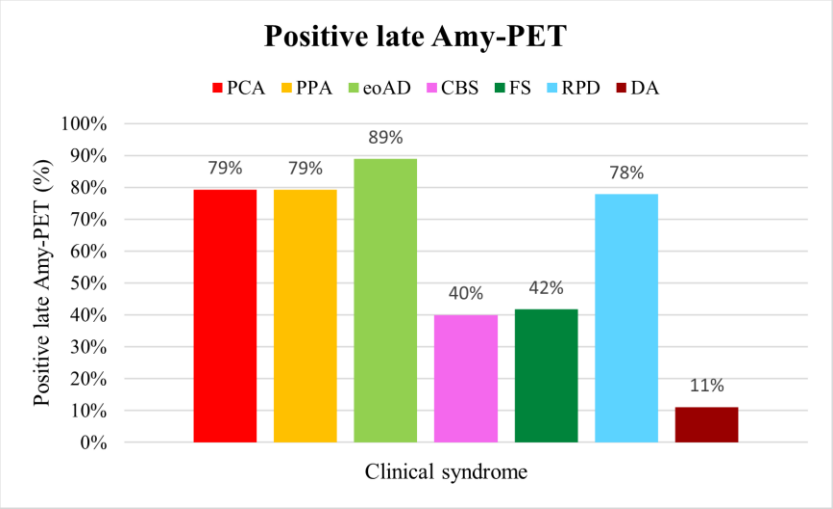

Figure 8 - Distribution of positive late Amy-PET in suptected clinical syndrome. PCA (posterior cortical atrophy), PPA (primary progressive aphasia), eoAD (early-onset Alzheimer's disease), CBS (corticobasal syndrome), FS (frontal syndrome) and RPD (rapid progressive dementia) and DA (atypical dementia)

In order to analyse the impact of Amy-PET in the diagnostic process, patients were classified into three categories according to the percentage of suspicion of presence of Alzheimer's disease by the neurologists at visit 1.

In 19 patients with a baseline poor suspicion of Alzheimer's disease, (i.e. estimate of being AD less than 50%), 7 patients (36,8%) had a positive Amy-PET. Whereas among 57 patients with a high suspicion of Alzheimer's disease (i.e. greater than 50%), 14 patients (24,6%) had negative Amy-PETs. Finally, among 6 cases with a doubtful suspicion (i.e. of 50% of being or not being AD), 3 patients had negative Amy-PETs and 3 patients had positive Amy-PETs.

In conclusion, in 27 out of 82 cases with available final diagnosis (32.9%) the result of Amy-PET scan did offer additional diagnostic information respect to clinical suspicion. The adding values of Amy-PET were also analysed respect to the clinical syndrome was found impact in CBS (80.0%), RPD (55.6%) and FS (50%). The lowest impact in eoAD and PCA. See table IV.

AMY-ITA PATIENTS				
pre PET AD presence	**patients**	**PET positive**	**PET negative**	**discordant rate**
< 50%	19	7	12	36,8%
>50%	57	43	14	24,6%
50 %	6	3	3	100.0%
total	82	53	29	32,9%
Early-onset Alzheimer's dementia (eoAD)				
pre PET AD presence	**patients**	**PET positive**	**PET negative**	**discordant rate**
< 50%	/	/	/	/
>50%	9	8	1	11,1%
50 %	/	/	/	/
total	9	8	1	11,1%
Corticobasal syndrome (CBS)				
pre PET AD presence	**patients**	**PET positive**	**PET negative**	**discordant rate**
< 50%	/	/	/	/
>50%	4	1	3	75.0%
50 %	1	1	0	100.0%
total	5	2	3	80.0%
Frontal syndrome (FS)				
pre PET AD presence	**patients**	**PET positive**	**PET negative**	**discordant rate**
< 50%	6	2	4	33,3%
>50%	4	2	2	50.0%
50 %	2	1	1	100.0%
total	12	5	7	50.0%
Rapid progressive dementia (RPD)				
pre PET AD presence	**patients**	**PET positive**	**PET negative**	**discordant rate**
< 50%	3	3	0	100.0%
>50%	6	4	2	33,3%
50 %	/	/	/	/
total	9	7	2	55,6%
Atypical dementia (DA)				
pre PET AD presence	**patients**	**PET positive**	**PET negative**	**discordant rate**
< 50%	4	0	4	0,0%
>50%	4	1	3	75,0%
50 %	/	/	/	/
total	8	1	7	37,5%
Posterior cortical atrophy (PCA)				
pre PET AD presence	**patients**	**PET positive**	**PET negative**	**discordant rate**
< 50%	2	0	2	0,0%
>50%	13	11	2	15,4%
50 %	/	/	/	/
total	15	11	4	13,3%
Primary progressive aphasia (PPA)				
pre PET AD presence	**patients**	**PET positive**	**PET negative**	**discordant rate**
< 50%	4	2	2	50,0%
>50%	17	16	1	5,9%
50 %	3	1	2	100,0%
total	24	19	5	25,0%

Table IV – concordance between AD initial clinical suspicion and AMY-PET result

At visit 3, the neurologists at each center assessed the change in clinical judgment following the FDG-PET and Amy-PET (early + late) results, giving a change rate of 60.8%, i.e., in 48 out of 79 patients. It should be noted that 4 patients have yet to be examined.

The rates of change in clinical judgement vary according to the initial clinical suspicion: RPD (87,5%), FS (83,3%), CBS (80%), DA (62,5%), PCA (60%), PPA (55,5%) and eoAD (11,1%).

Moreover, the rate of change of clinical judgement is also very heterogeneous between centers: Trieste and Prato changed judgement in 100% of cases; Perugia (83%), Genoa (80%), Pavia (67%) and Bolzano (50%) have high rates of change; while Parma (33%) and Padua (29%) have lower rates. See table V.

Distribution of late Amy PET positivity and change of clinical judgement in the different centres			
Centers	**Bolzano**	**Genoa**	**Padua**
total patients	4	10	24
clinical syndrome	FS(2), DA(1), PPA(1)	FA (1) PCA (3), PPA(6)	eoAD (7), FS(3), RPD(2), DA(2), PCA(1), PPA(9)
positive late Amy-PET	0/4	9/10	15/24
Positive late Amy-PET rate	0%	90%	62,5%
change of judgment	2	8	7
change of judgment rate	50%	80%	29%

Centers	**Perugia**	**Prato**	**Parma**
total patients	14	5	6
clinical syndrome	CBS (2), FS (2), RPD (4), PCA (3),PPA (3)	FS (1), RPD (1), DA (2), PPA (1)	eoAD (2),CBS (2), DA (1), PCA (1)
positive late Amy-PET	8	3	4
Positive late Amy-PET rate	57%	60%	66%
change of judgment	10/12 (not made visit 3 in 2 patients)	4/4 (not made visit 3 in a patient)	2/6
change of judgment rate	83%	100%	33%

Centers	Pavia	Trieste
total patients	12	8
clinical syndrome	CBS (1), FS (1), DA (2), PCA (6), PPA(2)	FS (2), RPD (2), DA (1), PCA (1), PPA (2)
positive late Amy-PET	8	6/8
Positive late Amy-PET rate	66%	75%
change of judgment	8/12	7/7 (not made visit 3 in a patient)
change of judgment rate	67%	100%

Table V- Distribution of late Amy PET positivity and change of clinical judgement in the different centres. PCA (posterior cortical atrophy), PPA (primary progressive aphasia), eoAD (early-onset Alzheimer's disease), CBS (corticobasal syndrome), FS (frontal syndrome) and RPD (rapid progressive dementia) and DA (atypical dementia)

4.4.4. CSF biomarkers and AMY-PET results: concordance or discordance

Only 17 of 83 patients underwent cephalospinal fluid analysis, which was positive in 8 cases and negative in 9 cases. While late amyloid-PET was positive in 9 patients. The concordance between CSF biomarkers and Amy-pet was present in 11 cases with 6 cases of positivity and 5 of negativity in both tests. The 6 discordant cases were divided into 3 cases with CSF biomarker negativity and late Amy-PET positivity and 3 cases with CSF biomarker positivity and Amy-PET negativity.

In the 3 cases of Amy-PET positivity and CSF negativity, there was one case of increased t-tau and p-tau, but with negative Aβ42 amyloid, in another two case Aβ42 amyloid was borderline, but t-tau and p-tau were negative.

In 3 cases the Amy-PET was negative, the CSF Aβ42 was below 500 ng/L and the CSF p-tau was negative.

4.4.5. Comparison of late visual and semi-quantitative analysis with DORIAN of the early phase (0-15 min) and late phase of 18F-FBB PET

66 out of 83 cases were analysed using DORIAN, as for the remaining cases the necessary images were unavailable or non-compliant.

In 56 out of 66 cases (84.8%) the whole brain analysis of all 4 methods used by DORIAN (SUVr, ELBA, TDr and RANK) showed concordance with the visual whole brain analysis of late Amy-PETs. Whereas in 10 out of 66 cases (15.2%) at least one of the methods disagreed with the visual interpretation. Analysing the distribution of the discordance of each method: SUVr discorded in 7 out of 10 cases (70%), ELBA in 4 out of 10 cases (40%), TDr in 2 out of 10 (20%) and RANK in 3 out of 10 (30%).

Considering that RANK uses all 3 methods of analysis (SUVr, ELBA and TDr), was the parameter of choice in evaluating the concordance between visual analysis and analysis done by DORIAN. So the concordance between the visual analysis and DORIAN analysis was 95,5%.

Of 3 patients, discordant between visual and Dorian analysis, the individual results of all 3 methods (SUVr, ELBA and TDr) dividing the whole brain into 10 areas were reviewed: LFA (left frontal area), LOA (left occipital area), LPPA (left posterior parietal area), LLTA (left lateral temporal area), LPPCA (left precuneus and post-cingulate area), RFA (right frontal area), ROA (right occipital area), RPPA (right posterior parietal area), RLTA (right lateral temporal area), RPPCA (right precuneus and post-cingulate area). Reviewing DORIAN's analysis the following data emerged:

- One patient had a negative RANK, SUVr and ELBA in whole brain analysis. Considering individual areas, there were some that were frankly positive in SUVr and ELBA
- One patient had a negative RANK, SUVr, ELBA and TDr considering whole brain analysis, only one area in TDr, right precuneus and post-cingulate area, resulted frankly positive
- One patient had a negative RANK and SUVr in whole brain analysis, but positive ELBA and TDr in all individual areas analysed

4.4.6. Correlation between different acquisition times (0-15minutes; 0-10 minutes; 0-5minutes) of the early phase of 18F-FBB PET

In the 66 cases analysed the concordance of the TDr method was analysed in the different acquisition times: 0-5 minutes, 0-10 minutes and 0-15 minutes. In the whole brain a significative TDr correlation, among the three different acquisition times, was obtained by the Spearman rank correlation coefficient (rS o ρ): rS 0,98 between 0-15 minutes and 0-10 minutes and between 0-10min and between 0-5. All with p<0,001. See figure 9.

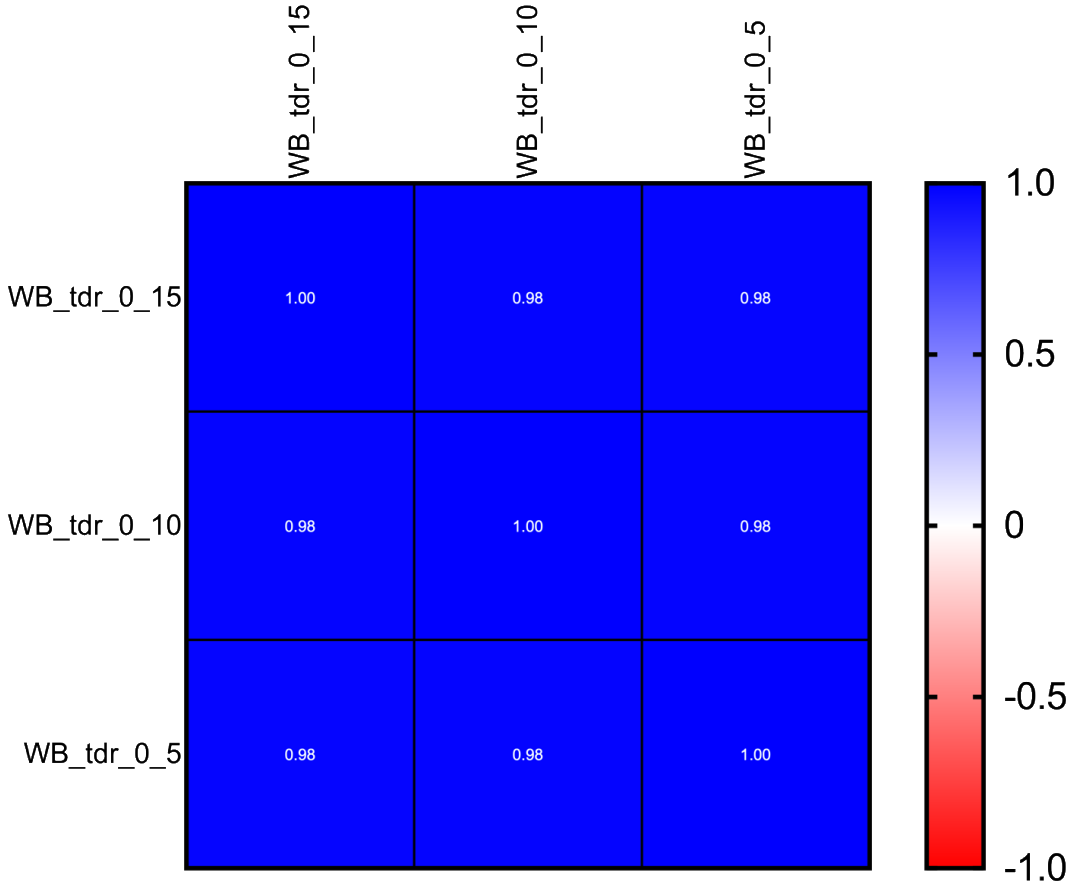

Figure 9 - Spearman correlation of TDr in whole brain. p<0,001. WB (whole-brain), TDr (time delayed ratio), WB_tdr_0_15 (TDr of whole brain in 0-15 minutes acquisition time), WB_tdr_0_10 (TDr of whole brain in 0-10 minutes acquisition time), WB_tdr_0_5 (TDr of whole brain in 0-5 minutes acquisition time)

TDr correlation between the three acquisition times was also performed area by area, showing a significant correlation. Table VI shows Spearman rank correlation coefficients. P-value <0.001.

Spearman's rank correlation of TDr			
areas	timeframes 0-5 vs 0-10 minutes	timeframes 0-10 vs 0-15 minutes	timeframes 0-5 vs 0-15 minutes
LFA	0,98	0,97	0,97
LOA	0,95	0,98	0,95
LPPA	0,95	0,98	0,93
LLTA	0,95	0,99	0,95
LPPCA	0,95	0,97	0,90
RFA	0,96	0,97	0,97
ROA	0,98	0,98	0,97
RPPA	0,97	0,96	0,96
RLTA	0,96	0,94	0,92
RPPCA	0,95	0,96	0,96

Table VI - Spearman's rank correlation of TDr among different acquisition times in 10 brain areas. P-value <0.001. LFA (left frontal area), LOA (left occipital area), LPPA (left posterior parietal area), LLTA (left lateral temporal area), LPPCA (left precuneus and post-cingulate area), RFA(right frontal area), ROA (right occipital area), RPPA (right posterior parietal area), RLTA (right lateral temporal area), RPPCA (right precuneus and post-cingulate area).

5. DISCUSSION

The study of amyloid deposits and neurodegeneration requires two different PET investigations, respectively with tracers for amyloid and with 18F-FDG, with high costs and double radio-exposure of the patient. In recent years, early phase Amy-PET, by assessing cerebral blood perfusion, has been proposed as surrogate markers of metabolism and therefore a alternative marker of neurodegeneration. The purpose of our study was to evaluate, by means of a visual and a semiqualitative analysis, the correlation between "perfusion" PET imaging, obtained with early frames 18F-FBB, and "metabolism" PET imaging, obtained with 18F-FDG, in a group of patients with atypical forms of cognitive impairment. The brain was ideally divided into eight regions for visual analysis. Two operators independently and randomly assigned a score from 0 to 3 depending on the degree of abnormality in the tracer distribution (FDG and early-FBB).

The first result obtained from the visual evaluation was the attribution of globally higher severity scores to 18F-FDG uptake deficits compared to early Amy-PET. This data could be explained by the ability of 18F-FDG to detect both flow anomalies and disconnection alterations, unlike the early-phase amyloid PET which provides information only on blood cerebral perfusion. According to the Spearman test, the scores attributed to each brain region in the PET images with 18F-FDG and in the early Amy-PET were statistically correlated. In accordance with the few studies available in literature, these results support the clinical utility of early Amy-PET images to obtain markers of neurodegeneration in patients with AD. Wilcoxon test demonstrated that there is no significant difference between the scores attributed to the occipital lobes in the images obtained with the two PET images; this is not valid instead for the frontal, temporal and parietal lobes. This discrepancy may depend on the fact that the FDG-FBB severity scores assigned to occipital lobes were less severe. In the frontal, temporal and parietal lobes, where the major distribution of neurodegeneration was found, FDG proved to be more sensitive than FBB-PET. The lower experience of nuclear medicine physicians in reporting results from early images of18F-FBB PET compared to the more classic and widespread reading of late

images with 18F-FDG may have influenced the attribution of severity scores. This may represent one of the limitations of this study.

According to the literature, the prevalence of AD differs between different clinical syndromes[23,24,26,30]. PCA, characterized by visual disorders, as visual agnosia, prosopagnosia, apraxia, ocular apraxia, alexia, simultagnosia, is frequently consequence of amyloid pathologic change, causing primary dysfunction of cortical posterior regions. PPA may be associated to AD, but moreover in one of its variant: the logopenic-PPA[30]. Core features of logopenic PPA are single-word retrieval difficulties, impaired repetition, verbal-memory difficulties, while fluency, grammatism and naming are spared. On the other hand, FS and CBS may be related to AD, but the prevalence is low and in good clinical practice other form of neurodegeneration must be excluded. The AD prevalence of PCA and PPA in our cohort is comparable to that reported in literature. In accordance with previous works, FS and CBS are the clinical syndrome less related to AD in our cohort, but for both variants amyloid pathologic change is more frequent than expected. Nevertheless, clinical and pathologic spectrum are various and differ between centers. It is possible to express these considerations:

- Amy-PET in eoAD almost always (88,9%) detects amyloid pathologic change;
- PCA and PPA are the prominent focal variants of AD; suspect amyloid change in these clinical variants was confirmed in high percentage of cases;
- FS and CBS are more frequently associated to AD in our cohort than in previous studies;
- RPD were often found to be the onset of AD;
- DA was frequently a non-AD related disease.

All patients of our study were enrolled with AD suspicion as relevant to justify indication to perform Amy-PET. This inclusion criteria can explain the higher prevalence in our cohort of less frequent AD atypical syndrome.

To analyse the impact of Amy-PET in the diagnostic process, two aspects were analysed:

- the discordant rate between the suspicion of the presence of Alzheimer's disease and the Amy- PET result. This aspect was analyzed by considering as discordant those cases in which the suspicion of the presence of AD prior to the performance of PET was less than 50% and the Amy-PET was positive or was greater than 50% and the Amy-PET was negative, while those cases in which the suspicion of the presence of AD pre-Amy-PET was 50% were considered discordant regardless of the result of the Amy-PET.
- The change in clinical judgement stated by the specialist during visit 3.

Clinical syndromes with more frequent discordance between pre-Amy-PET presence and the result of Amy-PET were CBS and RPD. While eoAD, PPA and PCA had a high concordance rate, this is justified by a higher presence of Alzheimer's disease association and a greater management capacity of neurologists due to a high number of patients examined in neurology centers.

CBS benefited most from the execution of Amy-PET: in 3 of 4 patients there was not concordance between expected presence and Amy-PET, in these cases a taupathy is the cause of the disease. While RPD was found to have a higher Amy-PET positivity than expected by neurologists. This could be due to an underestimation of the severity of the patient's symptoms by the patient and family members, resulting in a later intake of the patient characterized by a rapid worsening of a situation that had already been present for some time.

The rate of discordance between the suspicion of Alzheimer's disease and the Amy-PET result is 32.9%, which is lower than the rate of change of clinical judgement declared by the neurologists of the various centres at visit 3, which is 60.8%. This discrepancy between the two rates may be due to an interpretation of the change in judgment as positive when the hypothesis of suspected presence or absence of amyloid deposition is strengthened. In both analyses, the clinical syndromes with the highest rate of discordance or change in clinical judgment are RPD, CBS, and FS. In these clinical syndromes, the impact of performing Amy-PET remains greatest.

Moreover, the rate of change in clinical judgment is shown to differ from center to center. The significative differences in clinical judgment among centers may be explained by the heterogeneous prevalence of clinical variants.

Seventeen patients underwent both Amy-PET and CSF analysis, so strong conclusions can't be formulated. However, in two patients Amy-PET was diriment respect to CSF results: in one case the CSF level of Aβ42 was borderline and the CSF p-tau negative, in the other case the amyloid was negative, but the p-tau and p-tau were particularly high. Whereas in one patient with a dubious CSF but positive amyloid p-tau it was not possible to establish a clear clinical picture of the patient, partly due to the lack of further information as she was a patient from another centre. In this case, the coexistence of two causes of neurodegeneration, the genetic causes of AD or the suspicion of amyloid-positive cerebral angiopathy can be hypothesised.

Analysis with the DORIAN software makes it possible to perform a global, area-by-area semi-quantitative analysis of Amy-PETs by means of SUVr, ELBA and TDr. Nowadays, in clinical practice, the reading of Amy-PETs is mostly visual; there is no standardised semi-quantitative analysis.
Considering RANK, i.e. the method that analyses all three methods together, the study showed a concordance between the visual and semi-quantitative analysis of DORIAN in 95.5% of the cases analysed. Whereas when looking at the individual methods, there is a greater number of discordances, this is particularly the case with the use of SUVr alone, which discorded in 7 out of 66 cases (89.5%). This parameter has already proven to be less robust than the others in the literature[76]. Furthermore, in one case analysed, SUVr was completely negative both on the whole brain and area by area, while ELBA and TDr were positive and concordant with the visual analysis. On reviewing the images, it was noted that part of the cerebellum was missing and this made the analysis inconclusive, as the cerebellum is the reference area used in the analysis of SUVr; therefore, alterations or artefacts in the aforementioned region led to a failure of the analysis. The method with the highest concordance between visual and semi-quantitative analysis is the TDr with 96.6% of cases being concordant. A

particular case of discordance is a patient who was positive in the visual analysis but negative in all methods in the whole-brain, while in the area-by-area analysis he was frankly positive in the right precuneus and post-cingulate area in TDr method. In this patient it would be interesting to see the CSF analysis to see the level of Aβ42. In conclusion, DORIAN proves to be a good support tool for Amy-PET reading, especially when using TDr as one of the main parameters.

Finally, the TDr method of the DORIAN software, the only one to use early frames from Amy-PET, was used to analyse the presence or absence of concordance between TDr values acquired at three different times (0-15 minutes, 0-10 minutes and 0-5 minutes). This concordance was found to be statistically significant in both whole-brain and area-by-area analyses. This may possibly lead to a decrease of the acquisition times, especially important in non-compliant patients.

6. CONCLUSIONS

Preliminary data of AMY-ITA multicenter study confirm that early perfusion phase of Amy-PET is a valid surrogate of synaptic dysfunction, comparable to 18F-FDG PET in detection of neurodegeneration in atypical dementia on the basis of visual analysis. However, 18F-FDG resulted more sensitive than Amy-PET in graduating synaptic dysfunction, and this difference is statistically significant when synaptic dysfunction is moderate-severe. DORIAN semiquantitative analysis confirmed visual analysis result. For clinical practice, we can suggest Amy-PET early phase a useful tool for detection the presence or not of neurodegeneration in dementia. 18F-FDG PET remains superior for research purposes and in all cases quantitative scoring is needed. Amy-PET late frames resulted useful in atypical dementia even in case to confirm or exclude the suspect of amyloid deposition, as alternative instrument to CSF or, moreover, to support CSF analysis when inconclusive.

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