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Cerebral and CSF amyloid load, and recovery of semantic material in Alzheimer disease patients

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ABSTRACT

Key word: Alzheimer Disease, CSF biomarkers, flutemetamol-PET, Alzheimer Disease variants, SNAP

According to the new diagnostic criteria, the term "Alzheimer Disease" (AD) refers to a set of neuropathological changes that can be evaluated in vivo, rather than to a specific clinical symptomatology[1]. It is now widely accepted that β-Amyloid ($A\beta_{42}$) in the cerebrospinal fluid is a valid indicator of alteration of the pathophysiological state, associated with fibrillar deposits of cerebral β-amyloid[2]. Comparative studies between imaging and autopsy findings have established that amyloid PET images are a valid in vivo surrogate for deposition of fibrillar β-amyloid[3-10].

We analyzed the values of cerebral spinal cord (CSF) biomarkers in 40 patients with clinical diagnosis of AD. Two groups emerged: the first with both clinical and liquor biomarkers consistent with AD; the second was clinically in line with AD, but it was missing its pathognomic CSF biomarkers. At this point we asked ourselves about the nosological entity of the second group. All patients underwent flutemetamol-PET and the second group was dichotomically divided into two more groups based on the PET report. Schematically: Group 1: CSF + / PET +; Group 2: CSF- / PET +; Group 3: CSF- / PET-.

Our study then correlated the PET images through statistical software (spm12) in order to highlight any differences in cerebral β-amyloid accumulation. The first comparison was conducted between Group 1 and 2 revealing a significant accumulation of β-amyloid in the regions of the posterior cingulate gyrus. The posterior cingulate gyrus is involved in maintaining spatio-temporal orientation and memory functions, thanks to the connections with the parahippocampal cortex[11]. Involvement of the posterior cingulate gyrus is classic in patients with a typical clinical presentation of AD[11].

This result is also in agreement with the typical cerebral distribution of Aβ in AD (Braak and Braak stages)[12] and highlights instead the possibility of non-typical deposits in the second group, for which a different etiopathogenetic mechanism

from "ordinary" AD is hypothesized[13]. In support of this assumption, the results of the second comparison conducted between Group 2 versus Group 1, which showed a pattern of regional accumulation of cerebral β-amyloid in the regions of the frontal lobe, are explanatory. On the basis of this evidence, we hypothesized that the CSF- / PET + condition represents a clinical variant of the AD pathology defined in the literature as "frontal variant of the AD"[13]. Several studies have found that in the frontal variant of AD, the neuro-fibrillary tangle load (NFT) is about 10 times higher in the frontal cortex[13] than in the typical AD group. On the other hand, patients with typical AD showed a greater accumulation of NFT in the entorhinal cortex, cingulate gyrus and temporal cortex[13]. Both evidences are consistent with the results of our study.

Starting from the analysis of the neuropsychological tests carried out in AP patients behavioral and language alterations to the onset of illness have emerged, in addition to the memory impairment which is a pathognomonic sign of the typical AD. The typical AD refers to a pattern characterized by an early episodic memory loss followed by various combinations of deficits including attentional-executive deficit, language and visuospatial capacity deficits, which reflect the spread of the disease from the medial temporal lobe to other neocortical areas[14-17]. In contrast to this typical profile, the focal cortical variants of AD[18] present an atypical symptomatological picture (executive dysfunctions[19-20], deficits in design skills, behavioral abnormalities, impulsiveness, inattention to detail, inability to plan and language deficit[21]). Despite the serious alterations to the tests that investigate the functioning of the frontal lobe, the performance of neuropsychological tests were similar to the typical AD group. This suggests that severe frontal deficiency is the main neuropsychological feature on top of an otherwise typical AD profile[13].

Several studies suggest that the deposition of fibrillar Aβ explains at most, a small part of the clinical-anatomical heterogeneity of AD13. In fact, in the frontal AD variant an increase in tangles of tau fibrils but not of amyloid plaques has been observed[22-23].

It is now widely accepted that in AD the neurofibrillary lesions begin to accumulate in the limbic and temporo-parietal regions and only then would they progress to the frontal and occipital cortex. Thus the frontal lobes would be affected by neurodegenerative lesions typical of AD in a subsequent temporal sequence[12]. It is therefore possible that in the AD variants there is a focal deficit that is indicative of a selective, early and prominent vulnerability of some regions of the brain that are normally involved in pato

This result is also in agreement with the cerebral distribution typical of Aβ in AD (Braak and Braak stages)[12] and highlights instead the possibility of non-typical deposits in the second group, for which a different etiopathogenetic mechanism is hypothesized from that "typical" of AD[13]. In support of this hypothesis, the results of the second comparison conducted between Group 2 versus Group 1, which showed a pattern of regional accumulation of cerebral β-amyloid in the regions of the frontal lobe (Fig.2), are explanatory. On the basis of this evidence, we hypothesized that the CSF-/PET + condition represents a clinical variant of the AD pathology defined in the literature as "frontal variant of the AD"[13]. Several studies have found that in the frontal variant of AD, the neuro-fibrillary tangle load (NFT) is about 10 times higher in the frontal cortex13 than in the typical AD group. On the other hand, patients with typical AD showed a greater accumulation of NFT in the entorhinal cortex, cingulate gyrus and temporal cortex[13]. Both evidences are consistent with the results of our study.

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Aggregation of Aβ would be driven by the total flux of neuronal activity while tau aggregation would depend on trans-neuronal diffusion, generating neurodegeneration models that coincide with specific functional networks that eventually lead to specific clinical phenotypes (AD variants)[13]. A better understanding of the factors that drive the heterogeneity of these clinical phenotypes can provide important insights into the mechanisms of the disease and have direct implications on the diagnosis and management of patients with emerging disease-specific therapies[18].

Finally, in our study, the third and fourth comparisons were conducted between Group 1 and Group 3 and between Group 2 and Group 3 respectively. Both groups showed a significant pattern of accumulation of cerebral β-amyloid widespread almost in all brain areas. This result is not surprising, in light of the

fact that Group 3 probably configures the SNAP Group (suspected non-Alzheimer's pathophysiology), or a syndrome defined by normal levels of amyloid biomarkers (CSF- / PET-) but neurodegeneration patterns evident at MRI or FDG-PET[29] imaging study.

In fact, from 10% to 30% of clinically diagnosed ADs do not show neuropathological alterations of AD during an autopsy[30] and a similar proportion has Aβ[31] or CSF $A\beta_{42}$ levels normal[31-40]. Thus the multi-domain anamnestic phenotype of dementia is not specific. It may be the product of other diseases as well as AD[31]. To date, SNAP remains a not yet well-defined nosological entity. The clinical diagnosis of AD is often "incorrect" but there are significant differences with regard to clinical progress, genetic susceptibility and progression of the pathology, which have crucial implications for a precise and correct diagnosis, for clinical management and effectiveness of clinical trials on drugs[29]. SNAP is a very frequent condition in clinically normal subjects > 65 years and appears to be age-related. A study found that the frequency of SNAP was 0% in the age group between 50-60 years while it reached 24% around the age of 89[29]. However, the literature does not agree. The main controversy in the literature is whether SNAP is an indipendent pathological entity or can evolve into AD[41.] Some researchers believe that SNAP should be included as an integral part of the AD spectrum; if so, the pathogenetic explanation of the amyloid-centric models of AD and the concept of preclinical AD[42] are wrong and should therefore be reviewed. On the contrary, if SNAP is a different entity from AD, the amyloid-centric models of AD and preclinical AD[42] are completely consistent with current knowledge. In both cases, multiple studies have shown that the pathogenesis of SNAP is linked to the deposition of tau fibrils, which justify cerebral neurodegeneration; it would then be Aβ, even in small quantities, to act as the biological driver of taupathy, and cause the "spread" of tau in a widespread manner throughout the brain[43,44]. Therefore a better understanding of the factors that guide the clinical and etiopathogenetic heterogeneity of AD studied thanks to methods such as flutemetamol-PET can provide direct implications on correct diagnosis and prognostic precision in clinical practice. Furthermore, understanding the different nosological entities in study allows a better

stratification of the patients in the future trials and the management of emerging specific therapies for this disease.

Sommario

Parole chiave: Alzheimer Disease, CSF biomarkers, flutemetamol-PET, varianti della Malattia di Alzheimer, SNAP

Secondo i nuovi criteri diagnostici, il termine *Alzheimer Disease* (AD) è da riferire ad un insieme di cambiamenti neuropatologici valutabili in vivo, piuttosto che da una sintomatologia clinica specifica[1]. E' ormai ampiamente accettato che la β-Amiloide ($A\beta_{42}$) nel liquido cerebrospinale sia un valido indicatore di alterazione dello stato fisiopatologico, associato a depositi fibrillari di β-amiloide cerebrale[2]. Studi comparativi tra imaging e reperti autoptici hanno stabilito che le immagini PET amiloide sono un valido surrogato in vivo per la deposizione di β-amiloide fibrillare[3-10].

Abbiamo analizzato i valori dei biomarkers liquorali in 40 pazienti con diagnosi clinica di AD. Sono emersi due gruppi: il primo con clinica e biomarcatori liquorali concordi per AD ed il secondo con clinica concorde ma biomarcatori liquorali non patognomici di AD. A questo punto ci siamo interrogati sulla entità nosologica del secondo gruppo. Tutti i pazienti sono stati sottoposti alla flutemetamol-PET e il secondo gruppo si è diviso dicotomicamente in ulteriori due gruppi in base al referto PET. Schematicamente: Gruppo 1: CSF+/PET+; Gruppo 2: CSF-/PET+; Gruppo 3: CSF-/PET-.

Il nostro studio ha poi messo in correlazione le immagini PET con un software statistico (spm12), per evidenziare eventuali differenze di accumulo di β-amiloide cerebrale. Il primo confronto è stato condotto tra il Gruppo 1 e 2 ed è emerso un significativo accumulo di β-amiloide nelle regioni del giro cingolato posteriore. Il giro cingolato posteriore è coinvolto nel mantenimento dell'orientamento spazio-temporale e delle funzioni mnesiche, grazie alle connessioni con la corteccia paraippocampale[11]. L'interessamento del giro cingolato posteriore è caratteristico dei pazienti con una presentazione clinica tipica di AD[11].

Tale risultato è peraltro in accordo con la distribuzione cerebrale tipica di Aβ nell'AD (stadi di Braak e Braak)[12] ed evidenzia invece la possibilità di depositi in sede non tipica nel secondo gruppo, per il quale è ipotizzabile un meccanismo eziopatogenetico differente da quello "tipico" di AD[13]. A sostegno di tale ipotesi, sono esplicativi i risultati del secondo confronto condotto tra il Gruppo 2 versus il Gruppo 1, dal quale è emerso un pattern di accumulo regionale di β-amiloide cerebrale nelle regioni del lobo frontale. Sulla base di tale evidenza, abbiamo ipotizzato che la condizione CSF-/PET+ rappresenti una variante clinica della patologia di AD definita in letteratura "variante frontale dell'AD"[13]. Diversi studi hanno rilevato che nella variante frontale dell'AD, il carico di groviglio neurofibrillare (NFT) è circa 10 volte superiore nella corteccia frontale[13] rispetto al gruppo di AD tipico. D'altra parte, i pazienti con AD tipico hanno mostrato un accumulo maggiore di NFT nella corteccia entorinale, nel giro cingolato e nella corteccia temporale[13]. Entrambe le evidenze sono conformi ai risultati del nostro studio.

A partire dall'analisi dei test neuropsicologici effettuati in pazienti AP sono emerse alterazioni comportamentali e del linguaggio all'esordio di malattia, oltre al deficit della memoria a breve termine. L'AD tipico si riferisce a un pattern caratterizzato da una perdita di memoria episodica precoce seguita da varie combinazioni di deficit tra cui deficit attentivo-esecutivo, deficit del linguaggio e della capacità visuospaziale, i quali riflettono la diffusione della patologia dal lobo temporale mediale ad altre aree neocorticali[14-17]. In contrasto con questo profilo tipico, le varianti corticali focali di AD[18] presentano un quadro sintomatologico atipico (disfunzioni esecutive[19-20], deficit nella capacità di progettazione, anomalie di tipo comportamentale, impulsività, disattenzione per i dettagli, incapacità alla pianificazione e deficit del linguaggio[21]). Nonostante le gravi alterazioni ai test che indagano il funzionamento del lobo frontale, le prestazioni ai test neuropsicologici erano simili al gruppo AD tipico. Ciò suggerisce che il grave deficit frontale è la principale caratteristica neuropsicologica in cima a un profilo AD altrimenti tipico[13].

Diversi studi suggeriscono che la deposizione di Aβ fibrillare spiega, al più, una piccola parte dell'eterogeneità clinico-anatomica di AD[13]. Infatti nella

variante frontale dell'AD è stato osservato un aumento di grovigli di fibrille tau ma non di placche amiloidi[22-23].

E' ormai ampiamente accettato che nell'AD le lesioni neurofibrillari inizino ad accumularsi nelle regioni limbiche e temporo-parietali e solo in seguito progredirebbero verso la corteccia frontale e occipitale. Quindi i lobi frontali sarebbero interessati dalle lesioni neurodegenerative tipiche dell'AD in una sequenza temporale successiva[12]. È quindi possibile che nelle varianti dell'AD sia presente un deficit focale che è indicativo di una vulnerabilità selettiva, precoce e prominente di alcune regioni del cervello che sono normalmente coinvolte dalla patologia AD in una sequenza temporale successiva. Tale vulnerabilità sarebbe causata dalla primaria deposizione di tau a livello frontale[24-28]. D'altro canto la variante frontale dell'AD è caratterizzata da un processo patologico che non sembra rimanere limitato ai lobi frontali per molto tempo[18].

L'aggregazione dell'Aβ sarebbe guidata dal flusso totale dell'attività neuronale mentre l'aggregazione di tau dipenderebbe dalla diffusione trans-neuronale, generando modelli di neurodegenerazione che coincidono con specifici network funzionali che portano infine a specifici fenotipi clinici (varianti dell'AD)[13]. Una migliore comprensione dei fattori che guidano l'eterogeneità di questi fenotipi clinici può fornire importanti conoscenze sui meccanismi della malattia e avere implicazioni dirette sulla la diagnosi e la gestione di pazienti con terapie emergenti specifiche per la malattia[18].

Nel nostro studio, infine, il terzo ed il quarto confronto sono stati condotti rispettivamente tra il Gruppo 1e il Gruppo 3 e tra il Gruppo 2 e il Gruppo 3. Entrambi i gruppi hanno mostrato un significativo pattern di accumulo di β-amiloide cerebrale pressoché diffuso in tutte le aree cerebrali (Fig. 2). Tale risultato non sorprende, alla luce del fatto che probabilmente il Gruppo 3 configura il Gruppo SNAP (*suspected non-Alzheimer's pathophysiology*), ovvero una sindrome definita da normali livelli di biomarcatori amiloidei (CSF-/PET-), ma pattern di neurodegenerazione evidenziabili allo studio imaging MRI o FDG-PET[29] .

Infatti, dal 10% al 30% delle persone clinicamente diagnosticate come AD non mostrano alterazioni neuropatologiche di AD durante l'autopsia[30] e una

proporzione simile ha un livello di Aβ[31] o livelli liquorali di $A\beta_{42}$ normali[31-40]. Quindi il fenotipo anamnestico multi dominio della demenza non è specifico; può essere il prodotto di altre malattie così come l'AD[31].

SNAP rimane ad oggi un'entità nosologica non ancora ben definita. Spesso viene "erroneamente" posta diagnosi clinica di AD ma esistono differenze significative per quanto riguarda l'andamento clinico, la suscettibilità genetica e la progressione della patologia, le quali hanno implicazioni cruciali per una diagnosi precisa e corretta, per la gestione clinica e l'efficacia delle sperimentazioni cliniche sui farmaci[29].

SNAP risulta una condizione molto frequente nei soggetti clinicamente normali di età>65 anni e pare correlata all'invecchiamento. Uno studio ha rilevato che la frequenza di SNAP era dello 0% nella fascia di età tra 50-60 anni mentre raggiungeva il 24%, intorno agli 89 anni[29]. La letteratura non è tuttavia concorde.

La principale controversia della letteratura è se SNAP sia un'entità patologica a se stante o se possa evolvere in AD[41]. Alcuni ricercatori ritengono che SNAP debba essere inclusa come parte integrante dello spettro di AD; se così fosse la spiegazione patogenetica dei modelli amiloido-centrici di AD e il concetto di AD preclinico[42], sono sbagliati e sarebbero quindi da rivedere. Al contrario, se SNAP è una entità diversa da AD, i modelli amiloido-centrici di AD e di AD preclinico[42] sono completamente coerenti con le attuali conoscenze. In entrambi i casi, molteplici studi hanno evidenziato come la patogenesi di SNAP sia legata alla deposizione di fibrille tau, le quali giustificano la neurodegenerazione cerebrale; sarebbe poi Aβ, anche in minime quantità, ad agire come driver biologico di taupatia, e causare lo "spread" di tau in maniera diffusa in tutto il cervello[43,44].

Dunque una migliore comprensione dei fattori che guidano l'eterogeneità clinica ed eziopatogenetica dell'AD studiata grazie a metodiche quali flutemetamol-PET può fornire implicazioni dirette sulla corretta diagnosi e sulla precisione prognostica nella pratica clinica. Inoltre comprendere le diverse entità nosologiche in studio permette una migliore stratificazione dei pazienti nei futuri trials e la gestione delle terapie emergenti specifiche per malattia.

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that is typically characterized by impaired short-term memory and a progressive decrease in cognitive abilities[1]. From the anatomopathological point of view, it is associated with a continuing accumulation of senile plaques and neuro fibrillary tangles, which are responsible for neuronal death. Brain damage concerns at first medial temporal lobes[2], subsequently involves lateral temporal, parietal and frontal regions[3]. Several studies have investigated the sensitivity of specific biomarkers for Alzheimer's disease in order to discriminate the different stages of the disease and provide prognostic measurements[4,5]. Cerebral glucose uptake (GU), measured through fluorodeoxyglucose positron emission tomography (FDG-PET), and CSF-biomarkers are considered indicators for Alzheimer's disease in vivo. FDG-PET, in particular, detects not only cerebral metabolism, but also synaptic activity and is therefore reputed as an indirect measure of neuronal integrity<[6,7]. Based on FDG-PET imaging pattern, AD is characterized by a specific regional reduction of GU at the posterior cingulate level and in the parieto-temporal cortex [8,9]. Symptoms of AD essentially do not occur in absence of hypometabolism, whose amount typically reflects the severity of cognitive impairment[7].

Thanks to their diagnostic accuracy, CSF-biomarkers are also used in the clinical diagnosis of AD,and include markers for fibrillar neurodegeneration (such as total tau protein [Tau] and tau protein phosphorylated at threonine 181 [181p-tau]) and the deposition of βamyloid [$A\beta_{1-42}$]. Several studies have validated the use of low levels of CSF-$A\beta_{1-42}$as a marker for AD, although there is variability in the estabilishment of cut-off values for normality[10,11].

The relationship between pathology and cognitive ability has always been recognized as complex and non-linear. The concept of cognitive reserve (CR) was introduced to explain the gap between the extent of brain tissue damage and clinical symptoms observed on an individual level[12]. According to this hypothesis, AD patients with higher CR than those with lower CR require a greater accumulation of damage from brain pathology in order to show the same amount

of cognitive impairment[12]. This is supported by results from PET studies which suppose an inverse relationship between GU and years of school education (intended as an approximate measure for CR) in patients with different forms of neurodegenerative dementia[6.13].

Several studies have examined the role of CR in AD[12,14-17]; in detail, two of these used βamyloid-tracer PET[18,19]; on the other hand, as far as we know, to date no study has used liquor biomarkers as an indirect index of cerebral pathological load.

Chapter One

Alzheimer's Disease

1.1 History

The term "dementia" was introduced in medicine in 20 AD by Aulus Cornelio Celso, in the "De medicina". It used to indicate, in a generic way, a condition of alterated intelligence and behavior. Until the 18th century, however, the word remained confined to the social sphere. In 1797 Pinel used for the first time the term "dementia" referring to conditions that lead to the abolition of thinking skills. But it was one of his pupils, Esquirol, who in 1838 gave the term an early clinical identity ("clinical picture characterized by loss of memory, judgment and attention") and introduced the distinction between dementia - intended as acquired brain process - and congenital mental retardation (imbecility). Throughout the 1800s, however, no distinction was made between organic diseases and functional disorders in the psychiatric field, so that the term assumed a broad and generic meaning both in the popular and in the medical sense, and the demented patient was generically mistaken for the "madman".

The keystone in the history of dementia dates back to 1906, when Aloise Alzheimer, and later Perusini and Bonfiglio in 1910 in a more detailed way, described the clinical-pathological picture of a 51-year-old woman (Auguste D.) who had developed a progressive cognitive impairment with hallucinations and social incompetence. Cerebral atrophy, senile plaques and neurofibrillary balls were found in post-mortem examinations, in absence of a significant vascular compromise. The eponym "Alzheimer's disease" (AD) was suggested only in 1910 by Emil Kreplin in the 8th edition of his "Handbook of Psychiatry", where he used it to define a particular group of senile dementias portrayed by distinctive neuropathological alterations previously described by Alzheimer and Perusini; it's only afterwards that the eponym has extended more generally to all forms of primary degenerative dementia. Neverthless, up the second half of 1900 the

interest in diagnostic and clinical aspects was rather scarce and dementia was considered both the final common pathway of various conditions and an inevitable process connected to senescence. The increased availability of studying techniques for the functioning of the CNS in vivo and in experimental models, a clearer knowledge of neuropsychological processes, a better access to psychometric and psychological analysis tools, the advancement of methodologies and neuropathological knowledge, have led since the 1960s to a greater clinical characterization of dementias and their distinction from both psychoses in general and age-related changes in the cognitive functions. The introduction of defined clinical criteria (among the first, the DSM-IV criteria in 1994 and NINCDS-ADRDA criteria for Alzheimer's Disease in 1984) represented a further advancement in the clinical characterization of dementia, allowing a clearer and reproducible differentiation from other pathological conditions in which it is possible to find a cognitive decay.

1.2 Epidemiology

The prevalence of dementia, and in particular of AD, is constantly growing and today is one of the most important form of disability and social burdens in the world, representing, especially in societies where life expectancy has increased, the fourth cause of death in people aged over 65 years (about 3% of the total population) and being the cause of more than half of hospitalizations in retirement homes. It's estimated that 5 to 10% of the population between the ages of 65 and 74 is affected, while over 85 years of age 25 to 50% may be affected. There would therefore currently be around 20 million people suffering from dementia in the world, and this number is likely to escalate in future to 34 million in 2025 and 81 million in 2040. (Alzheimer society). Cognitive disorders of the elderly embrace a wide range of conditions: from the "benign" age-linked loss of memory, to the Mild Cognitive Impairment (MCI), up to the full-blown dementia. Of all the forms of dementia, the most common is AD (between 50 and 60% of total cases), followed by Vascular Dementia [(VaD) 20%], Lewy bodies Dementia

(15%) and lastly the rare reversible forms (5%). Table 1 shows the etiological classification of dementias.

DEMENZE PRIMARIE	CORTICALI -AD -FTD -Pick	SOTTOCORTICALI -LBD -Idrocefalo -Corea di Huntington -PSP -Degenerazione cortico basale
DEMENZE SECONDARIE	VASCOLARI	STATI CARENZIALI
		SOSTANZE TOSSICHE
	DISTURBI ENDOCRINO-METABOLICI -tiroide -insufficienza renale Disidratazione	PROCESSI ESPANSIVI INTRACRANICI -neoplasie -ematomi o ascessi cerebrali
	MALATTIE INFETTIVE E INFIAMMATORIE DEL SNC -meningiti e encefaliti -sclerosi multipla Creutzfeld-Jacob -AIDS	MISCELLANEA -traumi cranici Malattie cardiovascolari e respiratorie

Tab. 1. Etiological classification of dementias

1.3 Risk factors

AD is a multifactorial disease, in which several genetic and environmental risk factors seem to play an important role. The main responsible are, in order of importance, advanced age and familiarity (specifically for dementia and Down syndrome). On a genetic level, AD is divided into two forms: familial cases with early-onset Mendelian autosomal dominant inheritance (<60 years, Early-Onset Familial AD) and so-called "sporadic" cases with weak or non-existent familiarity, generally late onset (> 60 years, Late-Onset AD)[20,21]. It is also widely accepted that patients with Down syndrome are more at risk than the general population for developing AD after the age of 35[22], in relation to the fact that in trisomy 21 there is an increased production of β-amyloid due to over-expression of the amyloid precursor protein (APP), whose gene is located on the supernumerary chromosome[23]. Depression (in late-onset forms)[24] and moderate-severe head injury - which could cause axonal damage, hypoxia, increased

inflammatory response and oxidative stress[25,26] - also appear to be additional risk factors.

Role of education in the development of AD has long been debated, and the studies carried out still provide controversial results. The risk of developing other types of dementia appears to be higher in less educated subjects, but the association between AD and low level of education has not been confirmed[27].

Recent reviews have compared several studies that linked AD to cardiovascular risk factors, specifically hypertension, hypercholesterolemia, diabetes mellitus, obesity and cigarette smoking. Their results suggest an association between high levels of plasma LDL cholesterol and dementia, but they also show inconsistent results for the other listed risk factors[28]. Furthermore, a role of chronic cerebral hypoperfusion in the development of AD now seems certain. Numerous epidemiological studies have shown a reduction in the risk of AD in women who used post-menopausal hormone replacement therapy. To date it is believed that there is an effective risk reduction only in case of prolonged hormone replacement therapy (approximately 10 years) starting from the perimenopausal period[29,30]. Finally, several epidemiological studies conducted over the last 20 years suggested that prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs) could be a protective factor for AD. Unfortunately, long-term clinical trials with both non-selective NSAIDs and selective COX-2 inhibitors produced negative results. It is hypothesized that chronic use of NSAIDs could have AD risk-reducing effects only in the normal brain, where it inhibits the production of β-amyloid[31,32].

1.4 Pathogenesis

1.4.1 Amyloid cascade hypothesis

What Aloise Alzheimer in the early 1900s saw in the brain samples of the first AD case ever described were senile plaques, consisting mainly of amyloid substance[33]. It was only in 1984, however, that Glenner and Wong described for the first time the component of this substance, the β-amyloid (Aβ), developing the so-called amyloid cascade hypothesis[34]. Apeptidesβ derive from the altered

processing of APP, a transmembrane glycoprotein normally synthesized in the neuron and subsequently transferred on the membrane of the same cell. A soluble form of APP is released from the cell surface following proteolytic cleavage by the α-secretase enzyme (non-amyloidogenic pathway). The forming of Aβactually derives from the action of two other proteases, β-secretase and γ-secretase, which cut the APP in anomalous sites, respectively the first in the extracellular portion and then the second inside the double layer phospholipid of the cell membrane, thus releasing the abnormally long Aβ peptide (amylidogenic pathway). Cleavage by α-secretase inhibits cutting from the other two proteases, hence protecting cells from the accumulation of Aβ peptides[35,36]. Products from the hydrolysis of γ-secretase are respectively called $A\beta_{1-40}$and $A\beta_{1-42}$: they differ because the second one has two more amino acids in the C-terminal region, result of which is more easily attacked and therefore is the main responsible for the forming of amyloid fibrils[37]. The two variants are both present at low concentrations, and in soluble form, in plasma and liquor under physiological conditions, but in the brains of normal subjects the β-protein is completely absent (and this would depend on physiological mechanisms that allow the rapid elimination of the toxic molecule from the extracellular brain spaces). Aβ peptide becomes neurotoxic in cultured cortical cells when aggregated in βfilament structures[38], protofibrillar intermediates[39] and, above all, oligomers[40]. The latter are responsible for the final aggregation of the protofibrils and finally of the fibrils, which are the basis for the formation of senile plaques[41]. Therefore, according to this hypothesis, due to an increased production or a decreased disposal, the concentration of $A\beta_{1-42}$ increases progressively until it reaches a critical concentration of polymerization. The monomeric form of Aβ is not harmful, but its aggregated forms, in particular the oligomers, are toxic to synapses and neurons. In fact, the state of polymerization reached over the years triggers an oxidative stress in the neurons, which induces a degeneration accompanied by alterations of the cytoskeleton and leds to programmed cell death. Neurons appear to be particularly vulnerable to the attack by hydroxyl free radicals (ROS) for the following reasons: 1) their peroxidant glutatione content is low[42]; 2) their membranes are high in polyunsaturated fatty acids[43]; 3) brain metabolism requires

high amounts of oxygen[44]. The neuronal structures most involved in oxidative stress damage are mitochondria and cell membranes: mitochondrial DNA is, in fact, particularly sensitive to oxidative damage[45] and it has been shown that lipid peroxidation is the most important cause of membrane lipid depletion in AD[46,47].

1.4.2 Hyperphosphorylation of the tau protein hypothesis

As will be exstensively described below, one of the main anatomopathological aspects of AD is the presence of intracellular neurofibrillary tangles containing, as a major component, tau protein aggregates in a hyperphosphorylated state. The normal tau protein is part of the microtubule-associated proteins group (MAP), present in all the body especially within neurons. Its function is to modulate the stability of axonal microtubules, which are the internal "skeleton" of neurons and represent a fundamental transport system for nutrients and chemicals, such as neurotransmitters[48]. The gene that codes for the tau protein is expressed on chromosome 17 and there are 6 isoforms of theis protein, as a result of alternative splicing of some exons of the same gene[49]. All isoforms are often present in a hyperphosphorylated state in patients with AD: this causes microtubule disassembly and seizure of normal tau protein, MAP-1, MAP-2 and ubiquitin in helix filaments (neurofibrillary tangles). These insoluble structures damage the functionality of the cytoplasm and interfere with axonal transport, leading to neuronal death[50,51].

1.4.3 Inflammatory, cholesterol, and vascular hypothesis

Microglia, astrocytes and probably (although to a lesser extent) neurons, are implicated in the inflammatory process of AD. Aβ can activate the microglia; this leads to a higher expression of the major histocompatibility complex (MHC) of type II on the cell surface, together with the increased secretion of proinflammatory cytokines, such as interleukin 1β (IL-1β), interleukin 6 (IL- 6) and the tumor necrosis factor α (TNF-α), and chemokines, such as interleukin 8 (IL-8), the inflammatory macrophage protein 1 α (MIP-1α) and the chemo-attractant monocyte protein 1[52]. Aβ also triggers a phagocytic response in microglia and the expression of nitric oxide synthase (NOS), leading to neuronal damage. In addition, microglia can play a marginal role in the degradation of the

Aβ releasing the insulin-degrading enzyme (IDE): insulin, in fact, contributes to the extraneuronal accumulation of Aβ competing with the latter for the aforementioned enzyme[53].

In truth it is still not clear whether neuroinflammation is a primary cause or a secondary effect of AD genesis, as studies have shown that even normal astrocytes can secrete interleukins, prostaglandins, leukotrienes and other mediators of inflammation, as well as normal neurons[54].

The probable role of hypercholesterolemia as a modifiable risk factor for AD has already been described. Both production and clearance of Aβ are regulated by cholesterol. High levels of cholesterol increase the amount of Aβ in animal models of AD, and the drugs that lower cholesterol synthesis, on the other hand, cause their reduction[55].

Apolipoptrotein E (ApoE), produced by glial cells, is the most important brain protein for plasma cholesterol transport. It exists in three allelic isoforms ε2, ε3 and ε4: the latter is considered the most important genetic risk factor for the development of *Late Onset Alzheimer's Disease*[56]. The best hypothesis on the role of this isoform in AD regards its key role as mediator of the metabolism of the Aβ. ApoE indeed binds Aβ and is involved in its deposition and clearance; it's also necessary for the deposition of amyloid substance[57]. Furthermore, there is sufficient evidence showing that ε2 isoform of ApoE is a protective factor for AD[58]. Neuronal degeneration is enhanced by the production of inflammatory cytokines by microglial cells, usually primarily induced by Aβ polymers. Some of these (TNF-α, IL-1β, IL-6, IL-8, TGF-β) either directly and indirectly (by increasing the production of VEGF) are also able to induce angiogenesis. As early as 1907, Aloise Alzheimer had described artifacts characterized by endothelial proliferation and neovascularization. Patients with AD, compared with controls, have elevated levels of TGF-β and VEGF in CSF[59-61], implying a possible angiogenic mechanism in AD. Aβ plaques and neurofibrillary tangles could be angiogenic too. In vitro peptide Aβ has both pro[62,63] and anti-angiogenetic activity[64]; VEGF has indeed been found in plaques[65], and can be released from microvessels isolated from AD brains[66] and astrocytes in response to hypoxia[67]. Angiogenesis can therefore be constantly stimulated by inflammation and

hypoxia, leading to the formation of abnormal vessels, unable to supply nutrients and oxygen to degenerate areas. The basic growth factor of fibroblasts (another angiogenic factor) accumulates in areas of tissue damage together with neurofibrillary tangles Aβ[68]. The thrombin that builds up within the vessel wall in subjects with AD[69] enhances the effects of VEGF by inducing the expression of a receptor[70]. Therefore, it can be inferred that AD is mediated by pathological angiogenesis, suggesting that a series of angiogenic events contribute to the accumulation of Aβ and neuronal death. The abnormal vessels found in patients with AD are pathologically described as *cerebral amyloid angiopathy* (CAA): they have an increased diameter (microaneurysms), aberrant vascular branches, an enhanced proliferation of endothelial cells with thickening of the vessel wall, degeneration of smooth muscle cells and irregularities in the basement membrane[71]. These vessels are located in the hippocampus, in the middle frontal cortex and in the locus coeruleus - all regions involved in AD - and in leptomeninges. In this way vascular anomalies can worsen blood flow, reduce nutrient intake and allow the forming of inflammatory infiltrates. In AD the presence of CAA fluctuates between 70 and 97.6%[72]. The Aβ together with CAA, by causing microscopic bleeding in the cortex and white matter, today plays an important pathogenetic role in dementia. However, recent evidence from epidemiological, clinical, pathological and neuroimmaging studies considers cerebrovascular disease as an integral part of AD, thus giving rise to the*vascular hypothesis*, which involves all the main vascular risk factors. Initially it was believed that dementia could only occur in the presence of cerebral infarcts volume greater than 100ml[73]. In the light of more recent data, however, even smaller or microscopic infarcts can induce dementia[74-78]. The concept of *mixed dementia* refers to a wide range of conditions in which cognitive impairment can be attributed to AD- and vascular- type alterations [79,80] featured in several quantitative combinations[81]. Subcortical infarcts, for example, are often secondary to small vessels disease, which is characterized - from a pathological point of view - by the lipoialinosis of penetrating straight arterioles. Alterations in the vessel wall can lead to hemorrhages and infarcts, with micro bleedings due to the extravasation of small amounts of blood due to blood vessel fissures and

hemosiderin deposition. On T2-weighted MR imaging they appear as rounded lesions with a low but homogeneous signal. Besides the small vessel disease, microbleeds are now associated with *amyloid angiopathy*. However, the possible cognitive effects of microbleeds are not well known yet. A study has shown that microbleeds in patients with cerebrovascular disease have effects in particular on executive functions, but not on the intellectual ones or on other cognitive domains, including memory, language and visuo-spatial abilities[82]. Therefore it is not surprising that microbleeds may have neurocognitive effects, as it can be associated, from a pathological point of view, with tissue necrosis. In this way, depending on their location, size and number, they can cause localized or more extensive damage, and involve single or multiple cognitive domains. However, it's still important to understand whether microbleeds are an independent risk factor for the onset of cognitive impairment in vascular dementia, or whether they act in association with the typical lesions of AD. In conclusion, it can be deduced that an initial neuronal insult triggers an inflammatory response, resulting in the formation of new pathological vessels that allow toxic substances and immune cells to cross the already compromised blood-brain barrier (BEE), exacerbate tissue damage and perpetuate the inflammatory response. Therefore, this vicious circle can make the areas already affected by AD lesions even more vulnerable to the infiltration of cells of the immune system, leading them to neuronal death in a slowly progressive manner.

1.5 Genetics

Although most cases (approximately 98%) of AD occur as a sporadic form in old age, research carried out on patients with early-onset autosomal dominant forms of AD is the one providing valuable information on the pathogenesis of the disease. Mutations in 3 complete penetrance genes are found in almost all early-onset forms: APP, PSEN-1, PSEN-2 (presenilin genes, respectively on chromosome 14 and 1). On the other hand the APOE gene is the most important

genetic risk factor for the late-onset form, although it is neither necessary nor sufficient to explain all the characteristics of thepathology[83]. More recently, three new genes have been identified: CLU (clusterin gene), CRI and PICLAM-1[84].

The first mutations to be identified were those of the APP gene, located on chromosome2[85]: to date, 24 mutations of single nucleotides of this gene are known, each one leadin to an incorrect cleavage of the APP protein, although APP mutations justify less than 0,1% of all patients with AD[86]. Despite the fact that APP can code for several isoforms (the longest consisting in 750 amino acids) all the mutations present in AD are localized within a segment of 54 amino acids, adjacent to the sequence that codes for the peptides of Aβ. APP is proteolytically cleaved by β and γ-secretas, giving origin to two protein segments: $A\beta_{1-40}$and $A\beta_{1-42}$. All missense mutations influence APP processing because they are located in or near the encoding exons for Aβ (exons 16 and 17). In addition to the dominant ones, two recessive mutations (deletions of three nucleotides) of APP have been identified that determine the disease only in the homozygous form: E693Δ in a Japanese family, and A673V in another one. Although they're very rare, this suggests that new mutations may affect the APP gene, nonetheless it has been well studied, and this could explain at least some of the early-onset AD sporadic forms. Besides, the spectrum of mutations also extends to duplications, thus emphasizing the importance of dosing APP gene in AD; duplicated APP regions may contain several genes[87,89] or only APP[90] and are clinically linked to the early forms of AD with very extensive cerebral amyloid angiopathy[91]. APP duplications justify 12-18% of the dominant autosomal mutations in families with early-onset AD[92,93].PSEN1 and PSEN2 genes encode proteins, such as presenilin-1 and presenilin-2, belonging to γ-secretase complex (mutations damage cleavage with an increase in the $A\beta_{42}/A\beta_{40}$ratio). More than 180 mutations are known in PSEN1, causing AD with onset at the end of the third decade. The penetrance of these mutations is complete at the age of 60-65 years, which means that all carriers of this mutation develop AD. On the opposite, we know about 15 mutations of PSEN2, all of which with more variable penetrance than the previous ones.

Patients with mutations in PSEN1 and PSEN2 also tend to have a greater number of senile neocortex plaques and an $A\beta_{42}/A\beta_{40}$ ratio greater than in patients

with sporadic AD[94]. All three of these genes (APP, PSEN1 and PSEN2) support a common pathogenetic model for AD, which is based on the crucial role of Aβ. According to this hypothesis, therefore, the neurodegenerative process of AD is the consequence of an imbalance between the production and elimination of Aβ, suggesting that other genes involved in these mechanisms could contribute to the pathogenesis of the disease (Fig.1).

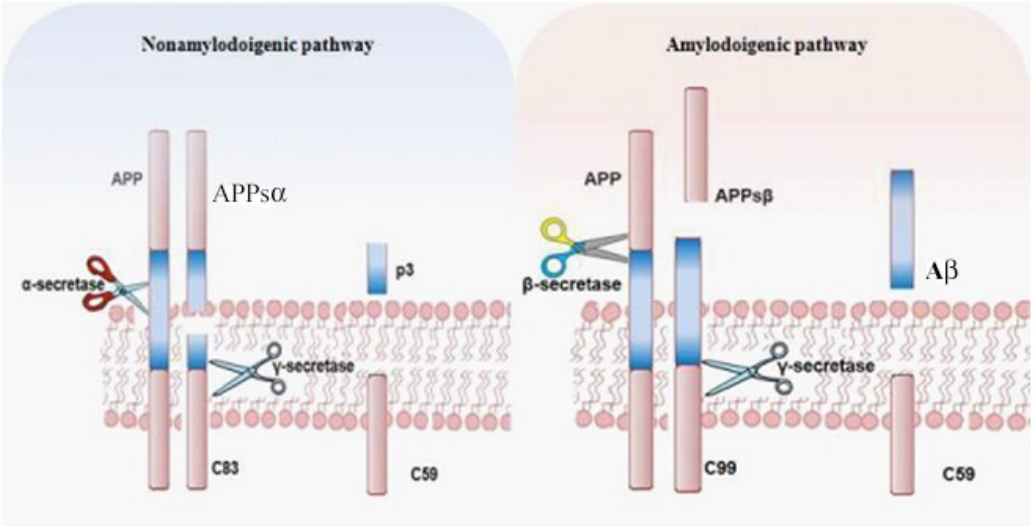

Fig.1 Non-amyloidogenic and amyloidogenic pathways

ApoE is a plasma cholesterol transport glycoprotein, whose gene is located in 19q13.2, and exists in three allelic forms (ε2, ε3 and ε4). In the CNS it is especially expressed in astrocytes, but also in microglia and neurons, allowing the transport of cholesterol and other lipids in the brain. It is conceivable that it acts as a neurotrophic factor in growth and repair during CNS development and trauma. Many epidemiological studies claim that ApoE ε4 is neither necessary nor sufficient for development of AD. ApoE ε4 has also been associated with coronary artery disease and is implicated as a risk factor for stroke and arteriosclerosis; finally, it presents a strong association with cerebral amyloid angiopathy, so it is currently believed that CAA contribution to AD is largely dependent on ε4[95]. ApoE may have isoform-specific abilities (ε2 <ε3ε4) to act as a chaperonin for Aβ and influence its metabolism, deposition, toxicity, fibril formation and removal from the brain, including tau-phosphorylation. Mutations

in the ApoE gene would now seem to be involved in the late and sporadic forms of the disease. The last genes identified in the pathogenesis of AD are CLU (encoding the clusterin), PICALM (encoding the phosphatidylinositol-binding clathrin assembly protein) and CR1 (encoding the complement C3b receptor). CLU is involved in Aβ elimination at BBB, as it increases its endocytosis within the glial cells. CR1 is the erythrocyte complement receptor for C3b protein and also participates in the elimination of circulating Aβ. CR1 mutations inhibit C3 functionality, inducing an increase in Aβ deposits and therefore neurodegeneration; this suggests a protective role of the complement towards AD. The role of PICALM is not yet known, but it could be involved in the processing of APP through endocytotic mechanisms and synaptic fusion.

In addition to the definitive identification of these 3 genes as actual susceptibility loci for AD, a synergy between APOE and PICALM has been identified, since they could participate to a common pathogenetic path[96-98]. (Fig.2)

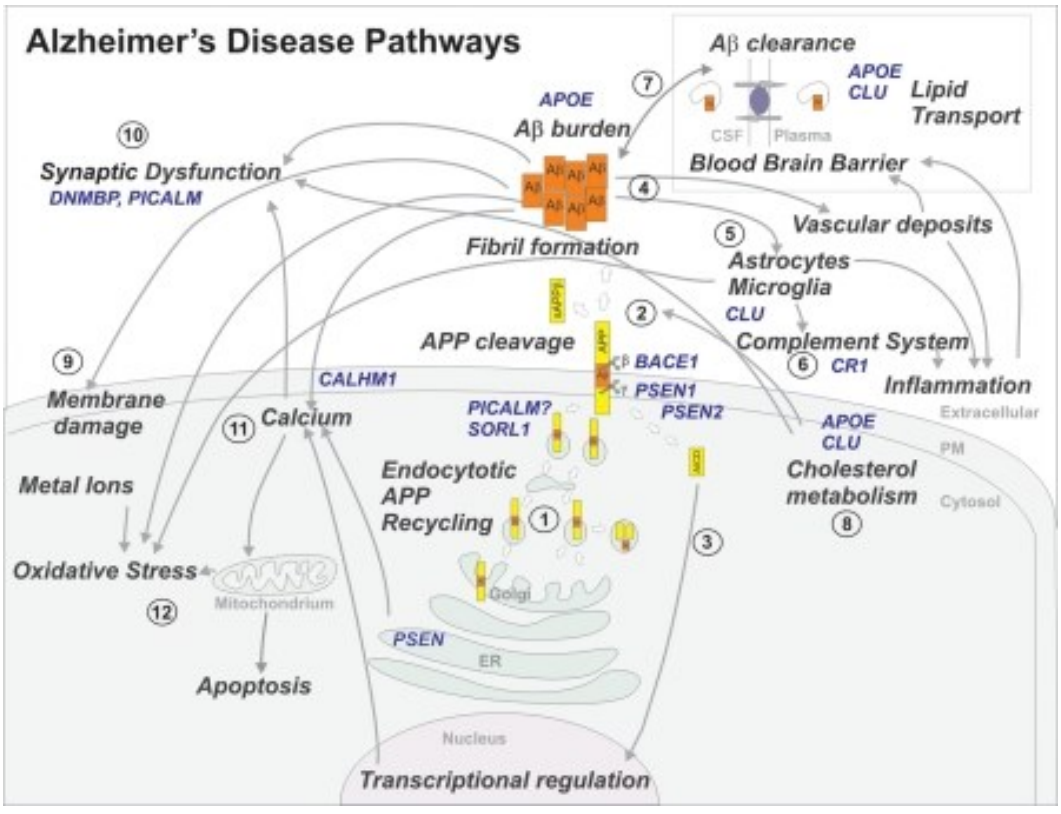

Fig. 2. Pathogenetic pathways:APP is synthesized in the endoplasmic reticulum and in the Golgi apparatus (1). Through the amyloidogenic pathway, the APP is cut from β-secretase (BACE1) andγ-secretase(PSEN) to generate the Aβ peptide and the intracellular

amyloid domain (AICD) (2), responsible for the transcription of several genes (3). In the retromer-mediated APP recycling pathway (1) APP is brought to the endosomes via SORL1. PICALM has a presumable role in the recycling of endocited APP (1). Aβ monomers aggregate into Aβ fibrils, causing amyloid plaques in the cerebral and vascular parenchyma (4). Aβ stimulates microglia and astrocytes, by activating complement system, the local inflammatory response and oxidative stress (5). CR1 is the C3b complement protein receptor and participates in the clearance of Aβ (6). Furthermore, by causing the increase in Aβ endocytosis in glial cells, CLU is involved in the clearance of Aβ through the blood-liquor barrier (7). APOE increases the formation of amyloid plaques depending on the conformational changes of Aβ. Clusterin (APOJ) and APOE are the main Aβ transport proteins in the brain (7). They play an important role in the cholesterol metabolism inside neuronal membranes (8) and high intracellular concentrations of cholesterol can boost the amyloidogenic pathway (2), which induces membrane damage (9). In addition, a defective cholesterol metabolism can influence synaptic dysfunction (10). Both PICALM and DNMBP are related to synapse (8). The anchoring of the Aβ oligomers to the membranes is further connected to the hypothesis of "calcium" in AD (11). Polymorphisms in the Ca2 + channel CALHM1 damages membrane permeability (11). Moreover, the PSENs influences the function of output channels for calcium, which accumulate in the cytosol. An excess of Ca2 + falls within the mitochondria, which worsens oxidative stress and apoptosis (12). *From "Current status on Alzheimer's disease molecular genetics: From past, to present, to future", Bettens et al, Hum Mol Genet 2010. Genes and risk factors considered "causative" of AD are marked in blue.*

1.6 Pathological anatomy

AD diagnostic certainty is possible solely with autopsy studies. On an external examination, the macroscopic picture is characterized by fronto-temporo-parietal atrophy, which however does not differ significantly from the changes found in the brain of patients of the same age not affected by dementia[99].

In addition to a variable thinning of the cortical gray layer, the marked atrophy of the hippocampus, which associates to the dilation of the adjacent temporal horn of the lateral ventricle, is suggestive for AD. Furthermore, the

entire supratentorial ventricular system appears to be expanded. Another characteristic is the loss of pigmentation of the locus coeruleus[100].

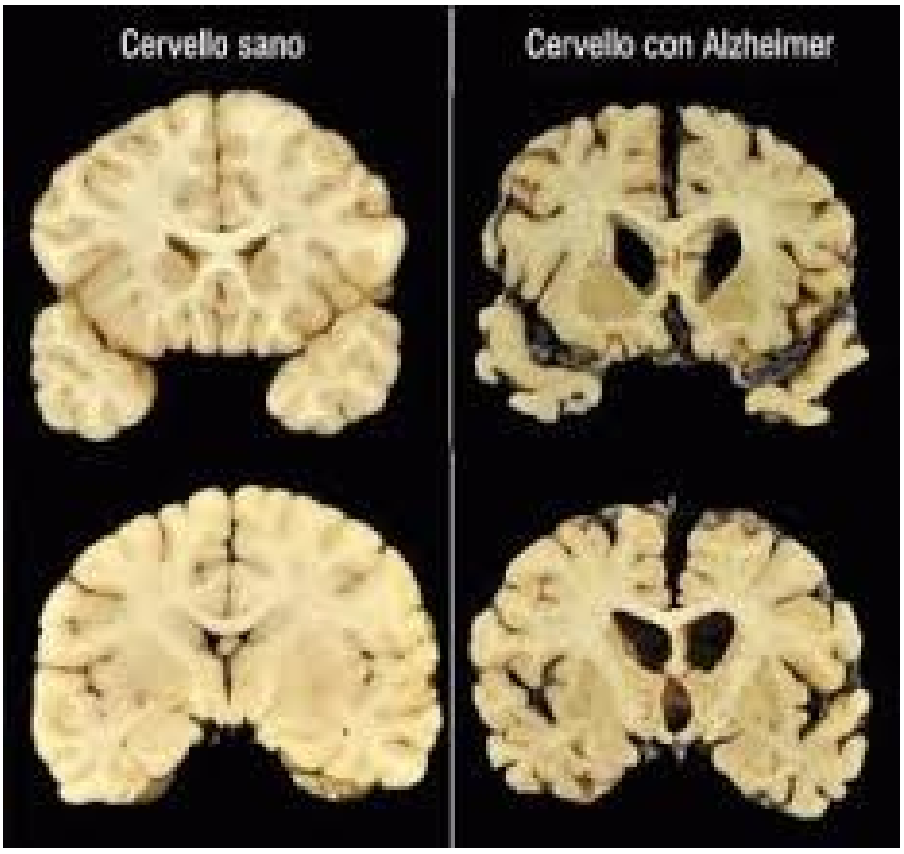

Fig. 3. Macroscopic anatomical-pathological picture of a normal brain (left) and a brain affected by AD (right)

At the microscopic level, characteristic alterations are found both in intracellular and extracellular sites. It is important to point out that in theory each of the microscopic alterations found in the AD can be identified, in different degrees, also in the brains of elderly subjects without cognitive decay. First of all in AD it is evident a substantial synaptic loss that correlates strongly with the degree of cognitive involvement, above all at the level of cerebral cortex, hippocampus, amygdala, basal nuclei. The mainly affected neurons are the cholinergic ones; as a mechanism of compensation an astrocytic proliferation can be found. In his pioneering article, Aloise Alzheimer noted the presence of two main microscopic lesions: *neurofibrillary tangles* and senile *plaques*(Alzheimer A, 1907). The neurofibrillary tangles are still considered the

cardinal microscopic lesion of the disease and a prerequisite for pathological diagnosis. These are anomalous fibrous inclusions within the cytoplasm of pyramidal neurons. Within the pyramidal neurons such tangles appear as thick parallel fibrils, which surround the nucleus and extend through the apical dendrite. When the tangle appears in a neuron with a more rounded configuration, the inclusion appears as a vortex of interwoven fibers, from which the name *globoid neurofibrillary tangle*. The tangle fibrils measure 10nm in diameter, occur in pairs and are organized in a helical bundle. The main constituent of the tangle is the tauprotein, which partecipates in the microtubule formation. The *tau* within the tangle is abnormally phosphorylated by two protein kinases, TPKI and TPKII, currently identified only in rats and cattle preparations, but not yet in humans[101,102]. The phosphorylated tau protein destabilizes the microtubule, preventing axonal transport and inducing neuronal death. On the other hand, TPKI can also phosphorylate the tau protein in the mitochondria, causing alterations in glucose metabolism and reducing the production of acetylcholine (Ach); reduced energy production and loss of signal transmission among cells can also induce neuronal death. Other components of the tangle are ubiquitin, cholinesterase and Aβ4. The neurons most involved are those of the lamina II in the entorhinal cortex, in the CA1 and subicular regions of the hippocampus, in the amygdala and in the deep laminae of the neocortex (superficial laminae III, V and VI). The extent and distribution of the tangles in the AD correlate with the degree of dementia and its duration[103,204]. However, tangles can also be identified in other morbid conditions, such as postencephalic parkinsonism, post-traumatic and boxing dementia, Niemann-Pick type C disease, ALS/Parkinsonism-dementia complex of Guam. The other AD key pathological lesion is the senile or neuritic plate, a complex structure formed by a central nucleus constituted by the amyloid Aβ of 4-kD, configured in beta sheets and organized in a radial beam. The nucleus is surrounded by a crown of dendrites and anomalous axons. At the periphery of the neuritic plaque it's frequent the presence of one or more microglia cells, less frequently reactive astrocytes. It's very common to find neuritic plaques in the brains of elderly individuals and subjects who do not show signs of cognitive decay. (Fig.4)

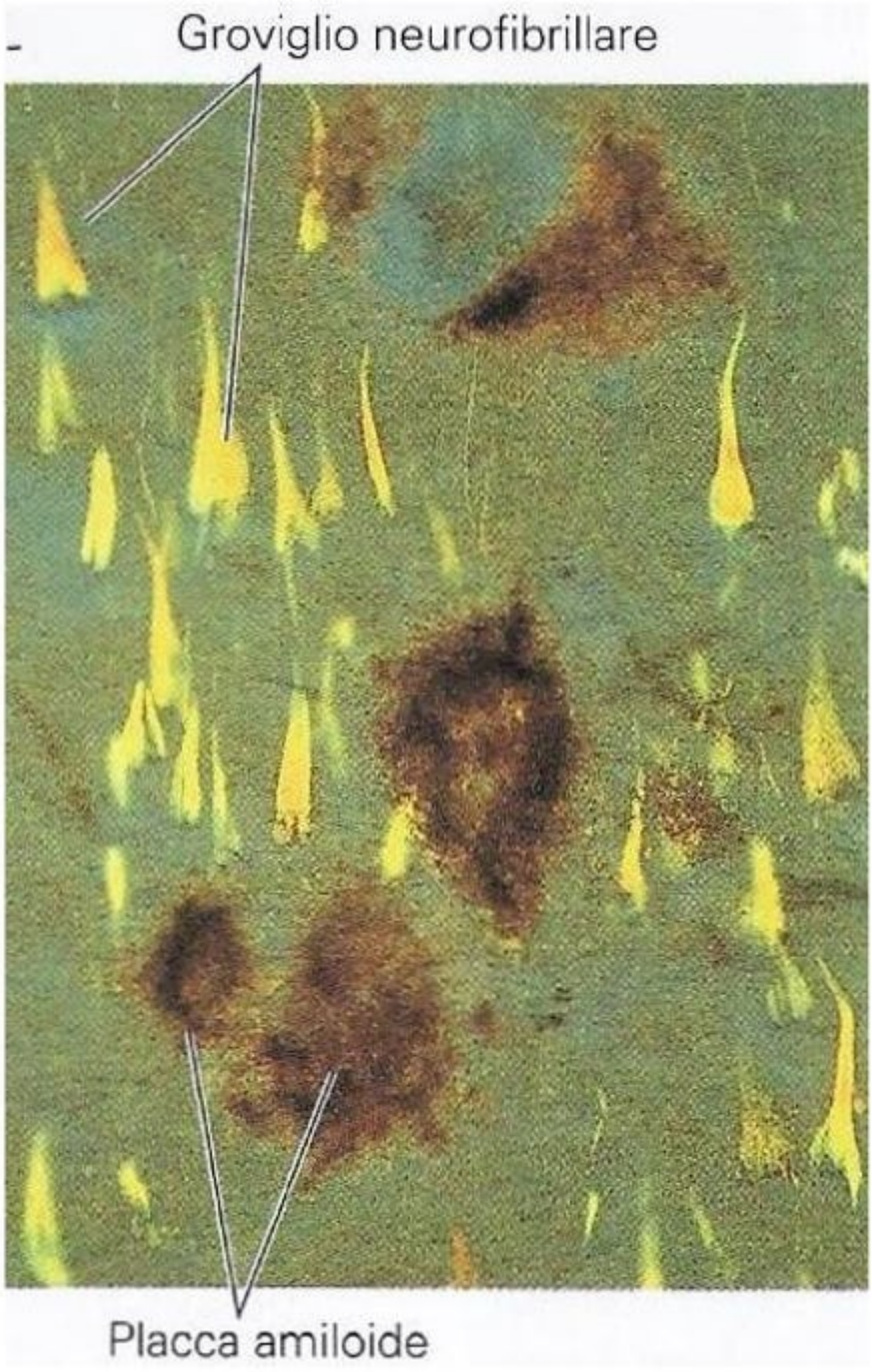

Fig. 4. Amyloid and neurofibrillary plaque

To date it is still not completely clear how these two lesions can explain neuronal death in patients with AD. The presence of senile plaques generally precedes indeed the onset of dementia, while neurofibrillary tangles appear simultaneously with the evolution of dementia. For this reason it is believed that these two lesions determine the neuronal death of AD[104]. The Aβ protein, other

than accumulating in the central nucleus of the neuritic plaques, tends to deposit itself in the wall of the small arteries and arterioles of the cerebral cortex and lepromeninges (*congophilic angiopathy*), but it does not seem to obstruct the vessel lumen nor does it seem to interfere with the function of these vessels. However, when the degree of vascular involvement is severe, there is a tendency for spontaneous rupture of the vessels with consequent cerebral hemorrhage mainly localized in the white substance of the frontal and occipital lobes. Bleedings are generally small and multiple, often microscopic; more rare is the case of larger lobar hemorrhages. In congophilic angiopathy, Aβ accumulates on the walls of cortical cerebral vessels and in the small arteries and arterioles of leptomeninges. The deposited amyloid has dye affinities similar to those of the senile plaque core. These piles of Aβ, as mentioned, do not appear to obstruct the vessel lumen nor interfere with the function of the vessels; however, when the degree of vascular involvement is severe, there is a tendency to spontaneous vasal rupture, which leads to a focal accumulation of blood at the level of brain tissues, especially in the white substance of the frontal and occipital lobes. These bleedings are often small and multiple, even microscopic; more rarely they appear as lobar hemorrhages, representing one of the few intracerebral complications of fatal ADs. One type of lesion still little known is the one constituting the so-called *granulovacuolar degeneration*, consisting of intraneural clusters of small vacuoles that measure from 2 to 4 μm in diameter, each containing a small, dense basophilic granule with a diameter of 1 μm. Little is known about the nature and significance of these lesions, as they have also been found in brain tissue samples of cognitively healthy individuals. Hirano*'s bodies are* intensely eusinophilic perineural lesions that are also found in individuals suffering from ALS/Parkinson-dementia complexes of Guam and in healthy subjects. They consist of parallel fibers of actin, tropomyosin, and vinculin that tangle in cross.

The set of lesions described accumulate in the brain over a period of several years. In 1991, Braak and Braak proposed a sequence of neuropathological progression, dividing the disease into 6 stages with an increasingly important brain involvement (Brakk and Braak[105] stages): in stages 1 and 2 there is a selective involvement of the entorhinal cortex and transentorinal by NFT; stages 3

and 4 show an increasing involvement of the limbic lobe; the last stages, 5 and 6, present the typical pattern of widespread neocortex involvement[106].

1.7 Clinical picture

AD is clinically characterized by an insidious cognitive impairment and progressive progression, which leads to the patient's complete dependence until his death. Depending on the age at onset, there are early-onset forms, before the age of 65, and later-onset forms, after the age of 65.

Dementia shows a wide variability of clinical pictures, even within the same etiological condition as in the case of AD. All patients have cognitive and non-cognitive symptoms, whose frequency depends on the stage of the disease. It should be noted that the person suffering from dementia maintains the character of historical and relational being and therefore the clinical manifestations depend on the interaction between neurobiological damage and numerous variables, such as the individual's personality, his history (for example the educational level), physical health, family and social network. Collectioning of anamnestic data represents the fundamental moment for a correct evaluation of the demented patient; in order for the story to be plausible it is very often necessary to question family members. A careful anamnesis can detect deficits in multiple cognitive areas, such as memory, language, praxia, visual-spatial abilities and criticism. In gathering history, it is necessary to investigate any difficulties in remembering recent events, in carrying out usual activities and procedures, in managing finances and business. The presence of episodes of topographical disorientation are of particular importance, as well as episodes of delirium, even short ones. It is useful to investigate the patient's behavior on particular occasions, such as a vacation or hospitalization; the occurence of topographic disorientation, agitation or a frank frame of delirium are suggestive of an underlying dementia.

The presence of non-cognitive symptoms must be evaluated with particular attention, both for their relevance from the diagnostic point of view, and for the impact on the quality of life of the patient and his family, and also because

they constitute one of the primary outcomes of the therapeutic intervention (pharmacological and otherwise) of dementia. Non-cognitive symptoms, which may already be present in the early stages of the disease, are heterogeneous, fluctuating and influenced by somatic and environmental variables; they also represent one of the major causes of institutionalization for stress and the caregiving burden of caregivers. Personality alterations are the most frequent non-cognitive symptom: apathy (70% of the cases), irritability (40%), disinhibition (30%), agitatio - which is a very composite disorder - ranging from persistent vocalization to aggression (60%); anxiety (50%); changes in mood are frequent: depressive symptoms (30-50%), euphoria (3-8%) and emotional lability (in 40% of cases); aberrant motor behavior (40%); psychotic symptoms (30-60%).

Of great importance in the etiological definition of dementia are the modalities of onset and progression of cognitive, non-cognitive symptoms and functional decline, even if there is some variability. A sudden onset of symptoms, with the presence of confusion, agitation, fluctuation of symptoms, must first of all lead to the exclusion of delirium; the presence of a triggering cause must therefore be sought (infectious diseases, heart disease, metabolic disorders, cerebral vasculopathy, drug or toxic poisoning, acute retention of urine, etc.). In AD the onset is typically insidious and progression is gradual; the initial symptoms are generally characterized by memory disorders, although speech or visual-spatial abilities disorders can sometimes be disease detectors.

The presence of depressive symptoms in the initial stages of the disease must focus on the possibility that alterations of cognitive functions are secondary to a depression and, generally, there is a personal remote anamnesis positive for depressive disorders. However, the differential diagnosis between dementia and depressive pseudodemence remains complex, just as the real meaning of pseudodemence stays uncertain, considering that about 50% of these patients develop irreversible dementia over five years[107]. The exact meaning of the depressive disorder that appears in the early stages of dementia is still uncertain; biological variables are probably involved, although personality aspects, disease insight, functional level, social and environmental variables are variously associated with the onset of depressive symptoms.

The presence, in the early stages of dementia, of a socially inappropriate behavior, associated with irritability, euphoria or apathy, sexual disinhibition, bulimia, which precedes the onset of memory impairment, is more common than fronto-temporal dementia. In VaD the onset is generally acute, sometimes associated with focal signs and symptoms. The course is typically stepped, with fluctuating symptoms. An acute onset and rapid progression of cognitive and functional deficits should lead to suspect a different etiology from AD, such as a stroke, a space-occupying mass, a metabolic, toxic or infectious cause.

The physical and in particular the neurological examination are fundamental in the patient evaluation. At the neurological examination, focal signs should be sought, since they would favor a vascular form or the presence of space-occupying masses (neoplasms, abscesses). Neurological signs of impairment of extrapyramidal and cerebellar pathways must also be searched. In AD, neurological examination can be negative or reveal the presence of signs of release, however not pathognomonic, in the most advanced phases of the disease.

1.7.1 Natural history

The average duration of AD is around 10-12 years; over time, the clinical picture undergoes important variations, with the succession and overlap of changes in cognitive performance, functional and behavioral framework, and the appearance of neurological and somatic problems. The first symptom of dementia is generally a slight memory loss that progresses gradually. In the early stages, memory loss tends to be more pronounced for recent events; the patient becomes repetitive, tends to get lost in new environments, forget commitments, can disorient over time. As the disease progresses, remote memory is invariably lost too and the patient is unable even to recognize his family members. It also shows alterations of the other higher functions: abstract thinking is impoverished, with reduced capacity for logical reasoning and conceptualization. Judgment is often early diminished, so that the patient shows a reduced job performance and can be unable to face and solve even simple problems related to his interpersonal or

family relationships. Emotional lability and personality change are manifested. The patient reports a progressive inability to perform tasks that are familiar and common to him. Apathy most commonly appears; the patient loses interest in the environment and for others, turning in on himself. Premorbid personality traits such as obsessive and compulsive attitudes, aggression, and paranoia are often exaggerated. In other cases, there is a change in personality, so that subjects normally controlled and measured become impulsive, intractable and sometimes even violent. This stage of disease is more easily detectable in younger subjects who still carry out work or professional activities; on the other hand, it can be overlooked in elderly patients or in those who don't generally perform intellectually demanding tasks. In some cases the disease occurs with an isolated aphasia or visual-spatial deficits. Aphasia is commonly fluent, with anomalies and paraphrasies. Apraxia can appear precociously, especially as difficulty using tools and clothing. At this stage the patient is generally managed by his own family and his relatives can be the first ones to notice an odd behaviour.

At an intermediate stage of the disease the patient becomes incapable of learning new information, and gets often lost in usually familiar environments. Remote memory is impaired, although not totally lost. The patient is at risk of falling and request assistance in the basic daily living activities; it is generally able to walk independently. Behavior is further compromised too. A complete space-time disorientation usually occurs.

In the advanced stages of dementia the patient is unable to perform any daily living activity; incontinence generally appears. Short-term memory is totally lost and the patient can become mutacic and akinetic. Dysphagia takes place and artificial feeding may be necessary. The risk of complications, such as malnutrition, dehydration, infectious diseases (especially pneumonia), fractures and bedsores, now becomes high. Lodging pneumonia is, to date, the most frequent cause of death for these patients.

1.8 Diagnosi

The diagnosis of dementia is essentially clinical or, in cases of certain diagnosis, post-mortem.

1.8.1 NINCDS-ADRDA diagnostic criteria

The criteria currently used are those proposed in 1984 by the *National Institute of Neurological and Communication Disorders and Stroke*, and by the *Alzheimers Disease and Related Disorder Association Work Group*(NINCDS-ADRDA[108]).

The NINCDS-ADRDA criteria foresee three different levels of diagnostic probability: defined, probable or possible (Fig.5). Neuroimmaging tests are envisaged as a criterion to support probable diagnosis, while the DSM-IV criteria (Fig.6), now replaced by the new DSM-V (Fig.7), make no reference to CT or MRI.

I. Probable Alzheimer's disease:
   a. Dementia established by clinical examination and documented by the MMSE, Blessed dementia scale or similar exam and confirmed by neuropsychological tests
   b. Deficits in two or more areas of cognition
   c. Progressive worsening of memory and other cognitive functions
   d. Onset between ages 40 and 90, most often after age 65
   e. Absence of systemic disorders or other brain diseases that could account for the dementia

II. Possible Alzheimer's disease
   a. Dementia syndrome, in the absence of other neurologic, psychiatric, or systemic disorders sufficient to cause dementia, and in the presence of variations in onset, in the presentation, or in clinical course
   b. Presence of second systemic or brain disorder sufficient to produce dementia, which is not considered to be the cause of dementia and
   c. A single, gradually progressive severe cognitive deficits identified in the absence of other identifiable causes

III. Definite Alzheimer's disease
   a. The clinical criteria for probable Alzheimer's disease and
   b. Histopathologic evidence obtained from a biopsy or autopsy

Fig. 5. NINCDS-ADRDA criteria

These criteria still play a certain importance today, because they include a gradualness in the diagnosis and the tools needed to deliver it (neuropsychological tests, rachicentesis, EEG, brain CT) and still allow a differential diagnosis with other pathologies, such as multi-infarct and other neurodegenerative diseases. Sensitivity of these criteria was assessed by several clinical-pathological studies at around 81% while specificity was at around 70%[110]. However, twenty years later,

a better knowledge of imaging, cerebrospinal fluid and AD genetic made a revision of the criteria necessary. Dubois et al. in 2007 and 2010 emphasized the need to revise the diagnostic criteria[111,112]. In 2011, the revised NINCDS-ADRDA criteria[111-113] were finally published. These new updated criteria maintain the nomenclature for *probability;* the definition of "*possible AD*" is not included, due to the incompatibility of this definition with the diagnostic criteria that are highly specific to AD. Atypical variants (including focal cortical syndromes[111])are not included. In the absence of highly specific biomarkers, *clinical* diagnosis is currently only probabilistic, even in case of a typical presentation[111]. In order to diagnose probable AD, the presence of the clinical criterion (A) and at least 1 or more of the supporting criteria (B, C, D or E) is required (Fig. 6)[111].

Panel 2: Diagnostic criteria for AD

Probable AD: A plus one or more supportive features B, C, D, or E

Core diagnostic criteria

A. Presence of an early and significant episodic memory impairment that includes the following features:

1. Gradual and progressive change in memory function reported by patients or informants over more than 6 months
2. Objective evidence of significantly impaired episodic memory on testing: this generally consists of recall deficit that does not improve significantly or does not normalise with cueing or recognition testing and after effective encoding of information has been previously controlled
3. The episodic memory impairment can be isolated or associated with other cognitive changes at the onset of AD or as AD advances

Supportive features

B. Presence of medial temporal lobe atrophy

- Volume loss of hippocampi, entorhinal cortex, amygdala evidenced on MRI with qualitative ratings using visual scoring (referenced to well characterised population with age norms) or quantitative volumetry of regions of interest (referenced to well characterised population with age norms)

C. Abnormal cerebrospinal fluid biomarker

- Low amyloid β_{1-42} concentrations, increased total tau concentrations, or increased phospho-tau concentrations, or combinations of the three
- Other well validated markers to be discovered in the future

D. Specific pattern on functional neuroimaging with PET

- Reduced glucose metabolism in bilateral temporal parietal regions
- Other well validated ligands, including those that foreseeably will emerge such as Pittsburg compound B or FDDNP

E. Proven AD autosomal dominant mutation within the immediate family

Exclusion criteria

History

- Sudden onset
- Early occurrence of the following symptoms: gait disturbances, seizures, behavioural changes

Clinical features

- Focal neurological features including hemiparesis, sensory loss, visual field deficits
- Early extrapyramidal signs

Other medical disorders severe enough to account for memory and related symptoms

- Non-AD dementia
- Major depression
- Cerebrovascular disease
- Toxic and metabolic abnormalities, all of which may require specific investigations
- MRI FLAIR or T2 signal abnormalities in the medial temporal lobe that are consistent with infectious or vascular insults

Criteria for definite AD

AD is considered definite if the following are present:

- Both clinical and histopathological (brain biopsy or autopsy) evidence of the disease, as required by the NIA-Reagan criteria for the post-mortem diagnosis of AD; criteria must both be present[23]
- Both clinical and genetic evidence (mutation on chromosome 1, 14, or 21) of AD; criteria must both be present

Fig. 6. Revised NINCDS-ADRDA criteria

1.8.2 American Psychiatric Association diagnostic criteria

Diagnostic criteria based on clinical findings are dictated by the American Psychiatric Association through DSM-IV in 1994, later modified in the DSM-V in 2013 (*American Psychiatric Association*)[109].

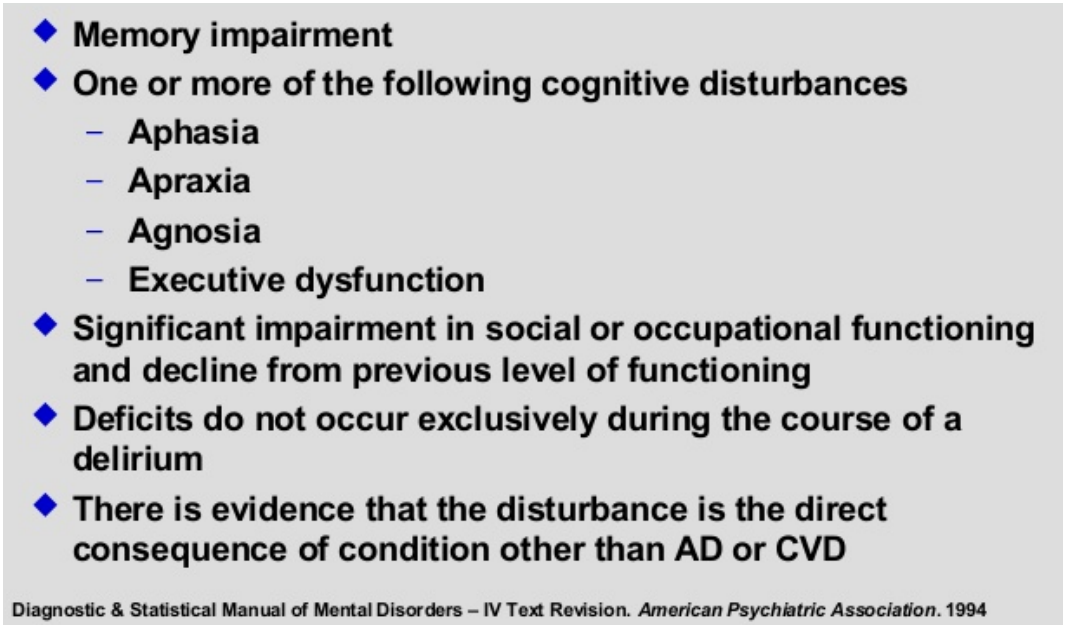

Fig. 7. DSM-IV diagnostic criteria

Diagnostic Criteria

A. The criteria are met for major or mild neurocognitive disorder.
B. There is insidious onset and gradual progression of impairment in one or more cognitive domains (for major neurocognitive disorder, at least two domains must be impaired).
C. Criteria are met for either probable or possible Alzheimer's disease as follows:

For major neurocognitive disorder:

Probable Alzheimer's disease is diagnosed if either of the following is present; otherwise, **possible Alzheimer's disease** should be diagnosed.

1. Evidence of a causative Alzheimer's disease genetic mutation from family history or genetic testing.
2. All three of the following are present:
   a. Clear evidence of decline in memory and learning and at least one other cognitive domain (based on detailed history or serial neuropsychological testing).
   b. Steadily progressive, gradual decline in cognition, without extended plateaus.
   c. No evidence of mixed etiology (i.e., absence of other neurodegenerative or cerebrovascular disease, or another neurological, mental, or systemic disease or condition likely contributing to cognitive decline).

For mild neurocognitive disorder:

Probable Alzheimer's disease is diagnosed if there is evidence of a causative Alzheimer's disease genetic mutation from either genetic testing or family history.

Possible Alzheimer's disease is diagnosed if there is no evidence of a causative Alzheimer's disease genetic mutation from either genetic testing or family history, and all three of the following are present:

1. Clear evidence of decline in memory and learning.
2. Steadily progressive, gradual decline in cognition, without extended plateaus.
3. No evidence of mixed etiology (i.e., absence of other neurodegenerative or cerebrovascular disease, or another neurological or systemic disease or condition likely contributing to cognitive decline).

D. The disturbance is not better explained by cerebrovascular disease, another neurodegenerative disease, the effects of a substance, or another mental, neurological, or systemic disorder.

Fig. 8 DSM-V diagnostic criteria

1.8.3 NIA-AA diagnostic criteria

Revised NINCDS-ADRDA diagnostic criteria havehighlighted a common framework regarding AD definition and its staging[112,113]. This is why the *National Institute of Aging and Alzheimer's Association* (NIA-AA), in 2018, has created a fresh approaching scheme; these new recommendations should be considered as research framework and not as diagnostic criteria or guidelines for diagnosis[114].

The need to read them as research framework derives from the common interest in validating and modifying data, before they can be used in usual clinical practice[114]

Definitions can be agreed on the basis of imaging and cerebrospinal fluid (CSF) biomarkers, which are widely used in research on AD and brain aging. The

scheme (labeled as ATN) recognizes three general groups of biomarkers according to the nature of the pathological process measured[114]. Biomarkers for amyloid plaques (indicated by "A") are represented by the cortical ligands in amyloid PET[115,116] or by low levels of CSFAβ42[117,118].Biomarkers for tau fibrils (labeled "T") are portrayed by levels in the CSF of phosphorylated tau (P-tau) and cortical tau ligands at PET[119,120]. Biomarkers for neurodegeneration or neuronal lesion (labeled "N") are the total tau at CSF (T-tau)[121], FDG-PET hypometabolism and MRI atrophy patterns[122,123]. The suitable mixture of biomarkers-image may be choosen based on the available resources; for example, when lumbar puncture and MRI are accessible but not PET, researchers can opt for CSF Aβ42 and P-tau respectively as A and T biomarkers and MRI as N biomarker.

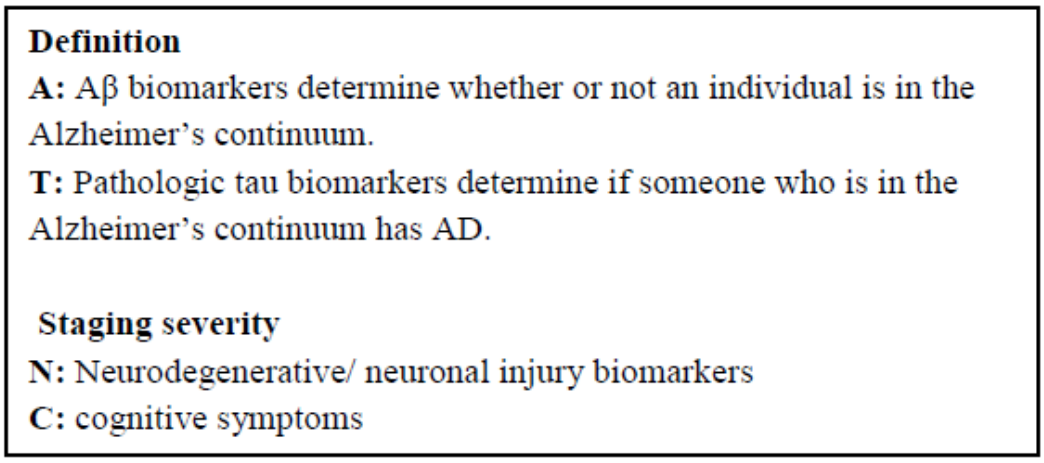

Fig. 9. Scheme for the identification of the components for the ATN classification

Each group of ATN biomarkers results in 8 different ATN "biomarker profiles": A + TN-, A + T + N +, etc. based on definitions. The ATN biomarker system assigns a subject to each of the three "biomarker categories":

1) individuals with normal AD biomarkers;
2) those in the continuum of Alzheimer's disease (divided into "Alzheimer's pathological change" and "Alzheimer disease");
3) those with a normal amyloid biomarker but with abnormal T or N, or both. This latter biomarker profile implies evidence of one or more

neuropathological processes other than AD[124] and has been labeled as "Suspected non Alzheimer's pathophysiology" (SNAP)[125]

An individual with biomarker evidence of Aβ deposition alone (abnormal PET or low CSF levels of Aβ 42 or low ratio 42/40) with a normal tau biomarker would be associated to the "Alzheimer's pathological change" label. The term "Alzheimer's disease" will be applied if biomarker evidence of both Aβ and pathological tau are present. "Alzheimer's pathological change" and "Alzheimer's disease" are not considered separate entities but as the earlier and later stages of the "Alzheimer's continuum" (an umbrella term that includes both Alzheimer's pathological change and Alzheimer's disease).

Neurodegenerative/neuronal biomarkers and cognitive symptoms, none of which are specific to AD, are used only as stage severity to avoid defining the presence of the Alzheimer's disease continuum[114].

<table>
<tr><th>ATN profiles</th><th colspan="2">Biomarker category</th></tr>
<tr><td>1. A-T-N-</td><td colspan="2">Normal AD biomarkers</td></tr>
<tr><td>2. A+T-N-</td><td rowspan="2">Alzheimer’s pathophysiology</td><td rowspan="4">Alzheimer’s pathophysiologic continuum*</td></tr>
<tr><td>3. A+T-N+</td></tr>
<tr><td>4. A+T+N-</td><td rowspan="2">Alzheimer’s disease</td></tr>
<tr><td>5. A+T+N+</td></tr>
<tr><td>6. A-T+N-</td><td colspan="2" rowspan="3">Non- AD pathophysiology</td></tr>
<tr><td>7. A-T-N+</td></tr>
<tr><td>8. A-T+N+</td></tr>
</table>

Fig. 10. The ATN classification

Of course the diagnostic pathway for patients with cognitive decline takes place in several stages. The Italian Society of Neurology has issued guidelines involving the general practitioner for initial screening and the neurologist for the diagnostic confirmation and differential diagnosis[195]. In the screening phase, a diagnostic suspicion is formulated and attempts are made to identify the causes that can induce cognitive impairment. A specific medical history, a complete physical and neurological objective examination, a functional

evaluation based on standardized scales of daily living activities (at least the *Instrumental Activities scale of Day Living:* IADL) must be performed and a cognitive evaluation based also it on the use of tests like the Mini Mental State Examination (MMSE). Basic laboratory tests would also be performed (complete blood count with formula, electrolytes, ESR, glycemia, azotemia, creatininemia, urine test, thyroid function test, blood levels of vitamin B12 and folate, serological tests for lue) to exclude a cognitive decay of metabolic or infectious origin, or caused by lack of vitamins. In special cases further laboratory tests may be performed: liver function, HIV-1 serology (AIDS-dementia complex), chest X-ray and blood gas analysis (chronic hypoxic syndromes), urinary metabolites of substances of abuse, urinary excretion of heavy metals, autoantibodies for the research of autoimmune diseases.

In the second phase diagnostic confirmation and differential diagnosis are performed. Although not strictly necessary for the diagnosis of dementia, every patient should have a complete neuropsychological evaluation, which involves the performance of a battery of tests that provides an indication on the severity of the cognitive deficit, the cognitive areas compromised and the eventual progression of disease. The presence of behavioral disorders such as depression will also be evaluated through standardized assessment tools such as the Neuropsychiatric Inventory (NPI). Alongside these purely clinical aspects, it is also possible to perform brain neuroimmaging tests, considered to be second level.

The standard Magnetic Resonance is the routine methodology as it allows accurate measurements of the three-dimensional volume of brain structures: in patients with AD, atrophy of the medial temporal lobe, especially of the amygdala, of the hippocampus and of the parappocampal gyrus is generally observed. Temporal atrophy causes enlargement of the Sylvian fissures, temporal horn, third ventricle and lateral ventricles (Fig.12). The entire limbic system is more or less atrophic, except for the anterior cingulate. The septal region and the posterior cingulate are also clearly atrophic. The atrophy of the precuneus or of the cortex of the parietal convexity is less marked, especially on the left. A disappointing aspect of the AD is that the typical pathological changes do not involve signal alterations compared to healthy brain tissue.

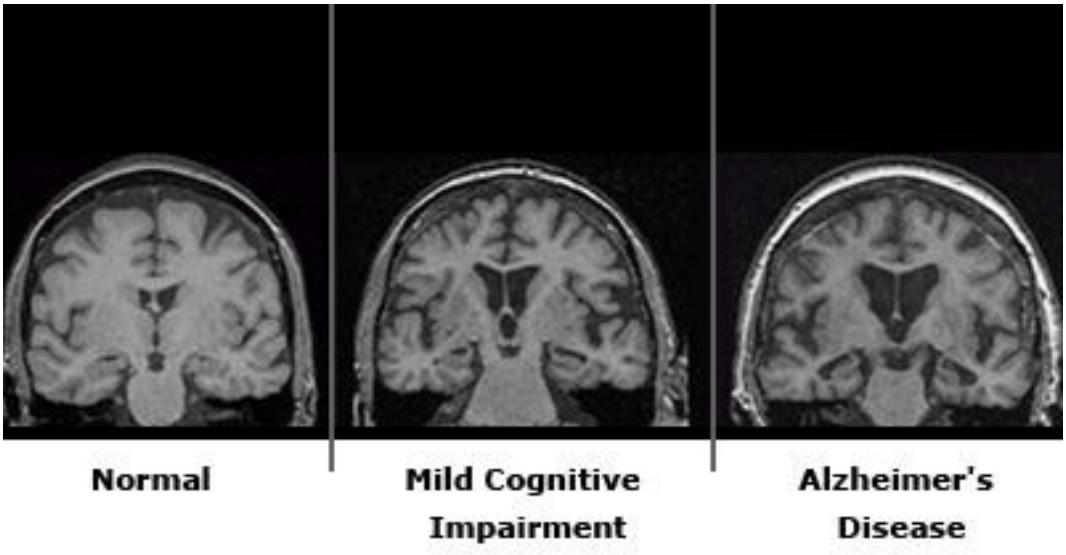

Fig.11 Brain MRI of a control versus MCI and AD: atrophy of the medial temporal lobe, above all of the amygdala, of the hippocampus and of the paraippocampal gyrus, with consequent enlargement of the sylvian fissures, of the temporal horn, of the third ventricle and of the lateral ventricles. The entire limbic system is more or less atrophic, except for the anterior cingulate.

However, recent studies are aimed at identifying radiological alterations affecting the white substance, in particular the corpus callosum. The atrophy of the corpus callosum in the AD represents the anatomical correlation of the Wallerian degeneration of the commissural nerve fibers, a consequence of the death of the pyramidal neurons of lamina III in the neocortex. Atrophy is localized mainly in the anterior regions (knee and anterior body) and posterior (isthmus and splenium). The posterior region subtends the parietal and temporolateral areas that, together with the temporomesiali structures, are those mainly and more precociously involved in the episodic memory circuits. The anterior region is instead responsible for the interhemispheric connections between the association prefrontal cortices, involved later in the AD and implicated in the monitoring of working memory information.

The involvement of the corpus callosum in the AD degenerative framework can be explained by a new pathogenetic hypothesis concerning the disease, which involves the myelinated fibers, affected by a process of myelin

interruption starting from the cortex towards the white substance[196,197]. According to this hypothesis, the fibers that during development myelinate later are those most susceptible to interruption and therefore they are the first to be affected by the disease, and vice versa. The corpus callosum contains fibers in the knee that myelinate later than those present in the splenium. Therefore the fibers of the posterior region, as directly connected with the temporo-parietal areas and consequently more precociously involved, undergo wallerian degeneration, while those of the anterior region, affected afterwards, undergo myelinic interruption.

Another test used in the diagnosis of AD is SPECT (single photon emission computed tomography): it reveals cerebral metabolism through the absorption of the radio tracer used, which is proportional to the cerebral flow. In SPECT studies there is generally a reduction in activity in the posterior parietal and temporal cortex: the severity of temporoparietal hypofunction in several studies has been correlated with the severity of dementia[198]. Furthermore, there is a reduction in regional cerebral flow especially in the hippocampus[199]. Today it is believed that clinically validated SPECT studies may show differences between AD patients and controls with a sensitivity and specificity of 80-90%[200].

Positron emission topography (PET) represents the first choice functional study in neurodegenerative pathologies. In particular, PET with 18-Fluoro-Desoxy-Glucose allows to map the cerebral glucose metabolism, which correlates with the neuronal synaptic activity. The altered uptake, at first in the posterior cingulate cortex and parietal regions, and subsequently in the temporal and prefrontal structures, corresponds to the progression of the neurodegenerative processes typical of the AD and consequently deafferented areas[201]. In patients with MCI and an AD-like hypocaptation pattern, a faster clinical progression towards AD is present, especially if they carry the ε4 allele of the APOE gene. Carriers of this allele show, however, a significant reduction in cerebral glucose metabolism in the second and third decades of life, when still asymptomatic[202].

With this type of study, a reduction in activity (in this case, of cerebral metabolism) at the level of parietal-temporal areas, of the posterior cingulate cortex and of the medial temporal lobe is evident. On average, sensitivity of FDG-PET reaches the 90% in identifying AD, but the specificity in differentiating it

from other types of dementia is lower[203]. In the last 10 years, PET tracers for fibrillar Aβ have also become widely available, which make the visualization of the disease in vivo possible. The most used amyloid tracer is the so-called Pittsburg compound B (PiB), which binds to Aβ plaques with high affinity. Several studies with PiB-PET have shown significant tracer retention in AD patients, compared with controls, especially in the frontal, parieto-temporal cortex, posterior cingulate cortex, occipital lobes, thalamus and striatum, compatible with the distribution pattern of amyloid plaques observed post-mortem[204-206]. Currently, other specific tracers for β-Amyloid have been marketed (18F-Florebetaben). From literature data[207], the use of amyloid-PET can be promising in two types of scenarios: a) to exclude Alzheimer's disease in specific cases where it is difficult to formulate a differential diagnosis, for example AD *versus* frontotemporal dementia (FTD); b) in order to create a good number of clinical stages that aim to improve treatments or prevention strategies for Alzheimer's disease, allowing patients to be enrolled in accordance not only with clinical and epidemiological criteria, but also with biological markers.

Not to be forgotten in the diagnosis of AD, the possibility of performing a liquor dosage of substances with a potential pathogenetic role in dementia, such as Aβ, Tau and p-tau.

It is established that the pathological process in the brain of AD patients begins more than a decade before the first symptoms208. Thanks to the description of the temporal dynamics of biomarker levels in relation to cognitive changes, it has been possible to realize a hypothetical model of the AD course based on the levels of liquor markers in the different stages[209]. The liquor markers used are the total tau protein (tau), which reflects the magnitude of the neuro-axonal degeneration, the p-tau, correlated with neurofibrillary degeneration, and the Aβ isoform made of 42 amino acids ($A\beta_{1-42}$), related to senile plaques.

Liquor levels of t-tau are 300% higher in patients with AD than in controls[210]. This marker is not considered specific for AD and elevated levels can be found in the CSF of patients with stroke or head trauma; levels even higher are found in patients with Creutzfeldt-Jakob disease[211]. Other types of dementia may

also present elevated t-tau values, since this is not considered a specific marker of neuronal and axonal degeneration.

In differential diagnosis with other dementias, p-tau could have a more important value, being more AD-specific. Elevated p-tau levels were found in patients with AD compared with others with fronto-temporal dementia (FTD) and VaD, dementia with Lewy bodies and Parkinson's disease with dementia[212]. P-tau made it possible to differentiate AD patients from FTD patients and dementia with Lewy bodies with a specificity of 92% and 64%, respectively[213]. However, a more important study carried out on patients with different types of dementia showed that a profile with typical AD biomarkers was also present in a substantial proportion of not-AD patients[241].

The levels of p-tau in the ante mortem liquor correlate with the amount of NFT and p-tau in the post-mortem brain. A recent study of cortical biopsies in patients with normotensive hydrocephalus showed a correlation between p-tau amounts in biopsies and p-tau levels inCSF[215]. The same study also showed that the presence of cortical amyloid plaques was associated with lower levels of $A\beta_{42}$ in the liquor: this could depend on the accumulation of$A\beta_{42}$ in senile plaques. Several studies, by using PiB-PET, have also found an inverse correlation between the amount of cerebral amyloid and the $A\beta_{42}$ in CSF[216]. The combination of these biomarkers can accurately distinguish AD patients from controls, with sensitivity and specificity above 80%[217]. Indeed, one study examined the performance of these markers in patients with AD at different ages and showed that despite the diagnostic accuracy decreases with age, the positive and negative predictive values of the combined biomarkers were established, so much so that markers could be used in elderly patients too[218]. Their long-term stability has also been evaluated: most studies have found that levels of $A\beta_{42}$ and p-tau remain unchanged over time, while data regarding total tau are inconclusive[219,220]. Some studies have not reported temporal alterations of t-tau in patients with AD, while others have reported its increase[221]. However, high levels of t-tau and p-tau seem to be associated with a more rapid progression of the pathology[222,223]. There are several methods for analyzing the three most important liquor markers for AD. One of the most common methods of analysis for $A\beta_{42}$ is

an ELISA test specially designed to measure Aβ, containing both the first and 42nd amino acid of the protein; there are also specific methods for the C-terminal end, which use N-terminal antibodies to measure fragments of free monomeric $A\beta_{42}$, but correlate well with a mass spirometry method based on SRM (Selected Reaction Monitoring) for total $A\beta_{42}$ [224].

The tau protein exists in several isoforms and can be phosphorylated on different residues. The most common ELISA test for t-tau identifies all the isoforms, regardless of their phosphorylation status.

The most widespread ELISA tests for p-tau measure phosphorylated tau at residues 181 or 231 and the diagnostic performance between the different methods is similar. A multiparameter method for the simultaneous measurement of $A\beta_{42}$, p-tau and t-tau has also been developed. Specifically, biomarkers values can be organized in a two-dimensional graph, with p-tau levels on the x-axis and the IATI index (INNOTEST^{TM} Amyloid Tau Index) on the ordinate axis, which allows to combine, for its turn, the values of$A\beta_{1-42}$ and p-tau with the formula of Hulsteart[225] (IATI =$A\beta_{1-42}$/(240 +1.18 tau)). The multicenter study conducted by Hulsteart et al. established cut-off values of 1 for IATI (IATI <1: suggestive for AD; IATI> 1: normal) and 61ρg / ml for p-tau. Several authors then used these parameters in their studies, finding sensitivity values up to 94% in the diagnosis of AD; the lowest levels of specificity were found, however, in the diagnosis of VaD (48%)[226]. Within the population affected by AD there is a subgroup recognized as SNAP (Suspected non-AD pathophysiology)[227], in which subjects have normal amyloid markers, but show signs of neurodegeneration different from AD. The combination of liquor biomarkers and neuroimmaging can help increase diagnostic accuracy, compared to the methods used individually.

A recent study has used RM, FDG-PET, t-tau, p-tau and $A\beta_{1-42}$ in CSF to predict the conversion from MCI to AD, concluding that the association of the methods can improve the prediction and the FDG-PET seems give the best prognostic information[228]. The combination of CSF $A\beta_{42}$,t-tau and p-tau, the study of subcortical MRI volumes and cortical thickness measurements also provide a better classification of MCI, AD and controls compared to biomarkers used

individually[229].

The $A\beta_{1-42}$/t-tau ratio increased the predictive accuracy for patients with MCI with both normal and abnormal hippocampal volumes on MRI who subsequently developed AD. MRI only increased diagnostic accuracy in patients with a normal CSF test[230]. Other studies have shown that t-tau, p-tau, $A\beta_{1-4o}$ and $A\beta_{1-42}$and medial temporal lobe atrophy on MRI provide independent information to discriminate between patients with AD and controls and also between patients with stable MCI and patients with MCI developing AD[231]. Amyloid imaging, unlike FDG-PET which is not very conclusive in MCI, allows the detection of the disease pattern from the prodromal bases, suggesting a timely intervention and the possibility to understand more deeply the underlying pathological processes in the Alzheimer'sdisease[232-234].The concordance between cerebral amyloidosis and CSF biomarkers has been evaluated in a few studies[235-245] using the Pittsburgh compound B PET tracer. Data collected from MCI and AD patients showed strong correlations between 11-C-PiB retention, levels of CSF biomarkers (especially$A\beta_{1-42}$), FDG-PET glucose metabolism and episodic memory. Regional brain differences have been observed as the disease progresses. The results obtained are not definitive and seem to suggest a complex model between pathological and functional markers with respect to the disease phase (MCI versus AD) and brain regions.To date, data in the literature assensing the concordance between cerebral amyloidosis measured by new PET tracers and CSF biomarkers are not known. In the evaluation of suspected encephalitis or when epileptic seizures are present, a useful examination is the EEG.

As for the differential diagnosis, its primary objective is to identify the dementias that can regress or not regress once the causes have been removed. An example is the so-called "depressive pseudo-dementia": the distinction in this case can be made through an accurate neuropsychological examination performed before and after specific therapy. It is also necessary to exclude dementias of vascular origin, diagnosable on the basis of clinical evidence and neuroimmaging of cerebrovascular disease. Excluding these two forms, the most probable diagnosis is that of a primary degenerative dementia, the most frequent of which is represented precisely by the AD.

1.8.4 Amyloid PET

In the last decade, advances in research in Alzheimer's disease (AD) and in molecular imaging have made it possible to detect in vivo the deposition of amyloid-beta (Aβ) in the human brain using positron emission tomography (PET). At the same time, progress has improved our understanding of Aβ as an important and therapeutically viable component of AD pathology.

While Aβ plaques are one of the pathological features that define AD, many healthy elderly people have elevated Aβ levels, as do patients with clinical syndromes other than AD dementia. The potential clinical utility of Aβ PET therefore requires careful consideration so that its role can be identified and inserted in the appropriate clinical context[342].

With the advent of carbon-11 (C-11) labeled as Pittsburgh Compound B (PiB), the "amyloid" Aβ has emerged as an important element in the form of a transformation of AD research that emphasizes the development of biomarkers, which could potentially facilitate the development of new drugs[343].

Intense efforts have been directed to assess the amyloid status of individuals with AD dementia as well as those with prodromal and preclinical stages of the disease, and the technology has been rapidly adopted around the world, although largely in specialized research centers. More recently, amyloid PET has been increasingly used in clinical trials for AD therapies. Since the short half-life of C-11 limits the routine clinical use of PiB due to the need for an in-situ cyclotron, amyloid-binding radiopharmaceuticals labeled with fluorine-18 with a longer half-life of 110 minutes, have been developed and marketed for wide availability[342]. They include flutemetamol F18, Florbetapir F18 and Florbetaben F18.

The prevalence of mixed-cause dementia increases with age and is frequently observed in clinical practice[344]. The integration of amyloid imaging into the clinical decision process can help narrowing a differential diagnosis and simplifying some of the complexities involved in evaluating patients with mild cognitive impairment and dementia. Although the identification of potential benefits, the AY (Amyloid Imaging Taskforce) concluded that an amyloid PET report will not constitute and is not equivalent to a clinical diagnosis of AD

dementia. Imaging is just one of the many tools that doctors should use judiciously to manage patients. PET amyloid imaging cannot replace an accurate history and examination. Indeed, history and physical examination are necessary to understand the clinical context necessary to incorporate imaging results into clinical decision-making[342].

The healthcare professional should keep in mind that amyloid imaging does not make a diagnosis of Alzheimer's disease and it doesn't ensure that a patient's cognitive impairment is due to AD's pathology. A limitation of amyloid PET against the diagnosis of AD dementia is the high prevalence of amyloid positivity in normal elderly subjects. Estimates of age-specific positivity rates for amyloid PET are <5% in those aged 50-60, 10% in 60-70, 25% in 70-80 and> 50% in people aged 80-90 [59, 60]. This prevalence associated with age means that the causality of Aβ for a patient's clinical syndrome cannot be established with amyloid PET alone, without considering the probability of previous positivity based solely on age. A dementia expert should consider the possibility, before prescribing amyloid PET, that the detection of amyloid linked to accidental age may not be related or relevant to a patient's presenting symptoms [342].

Another important element is that a positive amyloid scan can be present not only in AD, but also in other pathological conditions. For example, amyloid PET is frequently positive in dementia with Lewy bodies[345,346]. Furthermore, imaging detects both fibrillar amyloid in blood vessels (cerebral amyloid angiopathy) and interstitial fibrillar amyloid in plaques. Imaging is therefore not able to distinguish between amyloid angiopathy and parenchymal fibrillar plaques[347], and both are very common in the elderly, with or without dementia. Although they are generally associated with interstitial amyloid plaques, in rare cases the amyloid angiopathy can occur on its own[348]. Occasionally, amyloid angiopathy not accompanied by typical pathological features of AD can cause progressive dementia[349,350].

It's important to mention several clinical circumstances where amyloid PET is not expected to be useful: amyloid PET is not expected to detect the rare forms of AD in which ligand binding is greatly reduced due to unusual forms ofAβ[351,352].

The appropriate use of amyloid PET requires knowledge of all relevant results of clinical assessments, laboratory tests and imaging in relation to how each component of the accumulated tests should be weighed. Therefore, amyloid PET must be performed in the context of a global assessment undertaken by a doctor with experience in the evaluation of cognitive neurodegenerative disorders[342].

1.8.5 The use of FDG-PET in the diagnosis of AD

Numerous studies have found that neurodegenerative diseases may produce significant alterations in cerebral glucose metabolism, which are detectable thanks to PET-FDG. This allowed to study several of these neurodegenerative diseases such as cortical dementias and in particular AD[246]; it has been shown that with PET it is possible to carry out differential AD diagnoses even 2.5 years earlier than traditional diagnostic methods with an accuracy of 90%[247]. It is also possible to differentiate cases of dementia from other conditions such as normal aging and the presence of depressive disorders[248]. Furthermore, given that in various neurodegenerative conditions such as Alzheimer's and Hungtington disease there are silent and asymptomatic phases due to compensatory responses from the organ, the high diagnostic sensitivity of PET method allows to highlight the presence of functional alterations already in the preclinical phase of disease[249]. An important study[250] has observed that the use of glucose differs in subjects with Mild Cognitive Impairment (MCI) on the basis of age of onset and severity of symptoms: the lower the age of onset of symptoms and the greater the extension of glucidic hypometabolism (which may also involve the frontal lobe, the hippocampus, the cingulum and the nuclei of the base); moreover, the lower the score obtained at the MMSE (Mini Mental State Examination), the greater the probability of hypometabolism in the thalamus bilaterally and in the right caudate nucleus. In the same study[250] the difference is significant as regards age, disease severity and conversion time even in patients with MCI who convert to AD compared to those who do not convert: patients who do not convert have a hypometabolism limited to parietal regions and the right wedge but older ones may also not have metabolic deficits related to the disease;

finally, in patients showing disease progression within a year, hypometabolism is extended, involving the precuneus, the cingulate, the medial and lateral frontal region, the temporal and parietal regions. The hypometabolism of the entorhinal cortex has been significantly associated with the conversion from control to Mild Cognitive Impairment[251]. The importance of FDG-PET as a biomarker for Mild Cognitive Impairment and possible progression to AD has therefore been demonstrated: this functional investigation method is better than neuropsychological tests in predicting cognitive decline, in agreement with the fact that the manifestations pathological pathologies anticipate deficiencies related to test battery by some years[252]. The FDG-PET has been found to be the only biomarker capable, if used alone, of significantly increasing the conversion productivity value of AD (in association with age, ApoE, ADAS-Cog and education level data)[253]; the CSF and MRI data are not, however, significant results when these tests were used alone.

1.8.6 The use of CSF biomarkers in the diagnosis of AD

As previously mentioned, the NINCDS-ADRDA criteria for the diagnosis of AD now include the presence of CSF markers, useful for demonstrating the presence of pathophysiological processes of disease in progress; it is even possible to make a pre-dementia diagnosis. CSF markers used are the total tau protein (tau), which reflects the magnitude of the neuro-axonal degeneration, the p-tau, correlated with neurofibrillary degeneration, and the Aβ isoform made of 42 amino acids ($A\beta_{1-42}$), related to senile plaques. It is established that the pathological process in the brains of patients with AD begins more than a decade before the first finding of symptoms[254]. Thanks to the description of the temporal dynamics of biomarker levels in relation to cognitive changes, it has been possible to realize a hypothetical model of the AD course based on the levels of liquor markers in the different stages[255].

1.8.6.1 Biomarkers in MCI

Patients diagnosed with Mild Cogntive Impairment (MCI) have an intellectual deficit that, however slight, is more pronounced than that found in so-called physiological aging; moreover, it does not reach a global cognitive

involvement as in dementia, since the deficits found are sectoral and mild, without functional impact on the patient. This nosological category is therefore placed in an intermediate zone between normality and dementia.

The risk for patients with MCI to develop AD in a 4-5 year follow-up period is three times than for cognitively normal controls[256]. MCI is actually a heterogeneous disorder that can either progress to AD or other dementias, including VaD, frontotemporal dementia and dementia with Lewy bodies, or remain relatively stable and lead to normal cognitive decline of the elderly. The combination of low Aβ42 levels and high levels of t-tau and p-tau in liquor can accurately discriminate incipient AD from stable MCI[257]. Since the normal percentage of progression from MCI to AD is around 10-15%[258], it is important to have long follow-up periods to identify patients who are late in converting to AD. An important study found that the liquor levels of Aβ42, t-tau and p-tau in subjects with MCI can predict progression in AD with good accuracy after a mean follow-up period of 5.2 years[259]. The combination of t-tau and $A\beta_{42}$provided a sensitivity of 95% and a specificity of 83%. The population study was then re-evaluated after a much longer follow-up and established an average period of 9.2 years[260]. In fact, another group of patients with MCI had progressed to AD. Patients who progressed within 5 years had significantly higher levels of t-tau and p-tau compared to patients who progressed over 5-10 years, while $A\beta_{42}$ level was similar. A limitation of this study was however represented by the fact that the cut offs for the liquor markers had been established in the same cohort of patients used later to evaluate the diagnostic performance of the same markers to reveal the incipient AD.

In other stages the cut offs were determined by comparing patients with AD and controls and subsequently applied in MCI cohorts. Using this method, different combinations of $A\beta_{42}$, t-tau and p-tau were found that could predict the development of AD more than 5 years in advance with a sensitivity of 85-90% and a specificity of 71-82%[261]. The $A\beta_{42}$: tau ratio has also been used to predict progression to AD in patients with amnestic MCI (abnormal ratio of 79% of patients who developed AD)[262]. Of course, for the use of biomarkers, a standardization of analytical and pre-analytical factors is required, which also

demands harmonization among laboratories. In tests performed by the Alzheimer's Association's quality control program, the coefficient of variation among the laboratories that had contributed was from 15 to 25%.

1.8.6.2 CSF biomarkers in preclinical AD

The working group of the National Institute of Aging (USA) in 2011 asked how to define the presence of a preclinical phase of AD[263]. He then divided this phase into three separate stages: in the first, the patients have an amyloid pathology, defined by the presence of the latter at the brain FDG-PET or low presence of$A\beta_{42}$ in the CSF, with no signs of neuronal degeneration (normal brain MRI, t-tau levels in the standard); in the second stage the neuronal degeneration is evident and the patients have elevated levels of t-tau in the CSF or signs of neuronal damage in neuroimmaging tests; to be inserted in the third stage, on the other hand, patients must have mild memory impairments, but not so high as to be included in the criteria for MCI. To investigate the practical applicability of these guidelines, the authors applied them to a group of cognitively normal individuals: in this study only imaging markers were used, not CSF ones. After analyzing the results, they had to add two other categories: stage 0 (individuals with no signs of cognitive impairment and no pathological markers) and the SNAP stage, in which subjects have normal amyloid markers but show signs of neurodegeneration, and that they could be in the preclinical stage of a neurodegenerative process other than AD. The proposed criteria are far from being used by clinical practice and can constitute a basis for the search for new markers for preclinical AD. Studies have also been carried out to evaluate the presence of CSF biomarkers in the preclinical familial AD. The expected age for the symptoms was calculated basedir on the onset age of their parent's AD. The carriers of an autosomal dominant mutation showed significantly elevated levels of CSF t-tau and plasma $A\beta_{42}$ 15 years before the expected onset of symptoms, while the levels of CSF $A\beta_{42}$ were reduced 10 years before onset[264]. Longitudinal studies then have correlated the basic levels of biomarkers in cognitively normal individuals with the decline of cognitive functions or the development of MCI or AD. The CSF levelsof $A\beta_{42}$ isolated[265] or in combination with t-tau[266] or p-tau[267] were associated with future development of cognitive impairment in cognitively normal patients at

the start of the studies. Although multiple investigationss on the subject have been completed, there still aren't prospective long-term studies that have analyzed whether CSF markers may predict the development of AD in healthy elderly subjects within 10-20 years.

1.8.6.3 Biomarkers in AD

CSF levels of t-tau are 300% higher in patients with AD than in controls[268]. This marker is not considered specific for AD and elevated levels can be found in the CSF of patients with stroke[269] or head trauma[270]; levels even higher are found in patients with Creutzfeldt-Jakob disease [271]. Other types of dementia may also present elevated t-tau values, since this is not considered a specific marker of neuronal and axonal degenerationz[272].

In differential diagnosis with other dementias, p-tau could have a more important value, being more AD-specific[273]. Elevated p-tau levels were found in patients with AD compared with others with fronto-temporal dementia (FTD) and VaD[274], dementia with Lewy bodies and Parkinson's disease with dementia [275]. P-tau made it possible to differentiate AD patients from FTD patients and dementia with Lewy bodies with a specificity of 92% and 64%, respectively. However, a more important stage performed on patients with different types of dementia showed that a profile with typical AD biomarkers was present, even in a substantial part of the non-AD patient[276]. The levels of p-tau in ante-mortem CSF correlate with the amount of NFT and p-tau in the post-mortem brain[277,278]. A recent study of cortical biopsies in patients with normotensive hydrocephalus showed a correlation between p-tau amounts in biopsies and p-tau levels in CSF. The same study also showed that the presence of cortical amyloid plaques was associated with lower levels of $A\beta_{42}$ in the liquor: this could depend on the accumulation of $A\beta_{42}$ in senile plaques. Several studies, using BiP-PET, also found an inverse correlation between the amount of cerebral amyloid and CSF $A\beta_{42}$[280,281]. The combination of these biomarkers can accurately distinguish AD patients from controls, with sensitivity and specificity above 80%[217]. Indeed, one study examined the performance of these markers in patients with AD at different ages and showed that despite the diagnostic accuracy decreases with age, the positive and negative predictive values of the combined biomarkers were

established, so much so that markers could be used in elderly patients too[282]. Their long-term stability has also been evaluated: most studies have found that levels of $A\beta_{42}$ and p-tau remain unchanged over time[283,284], while data regarding total tau are inconclusive[285]. Some studies have not reported temporal alterations of t-tau in patients with AD, while others have reported its increase. However, high levels of t-tau and p-tau seem to be associated with a more rapid progression of the pathology[286,287].

1.8.6.4 Measurement of CSF biomarkers

There are several methods for measuring CSF biomarkers. One of the most common methods of analysis for $A\beta_{42}$is an ELISA test specifically producedtomeasure$A\beta_{42}$, containing both the first and the 42nd amino acid of the protein: there are also specific methods for the C-terminal end, which use antibodies N-terminals to measure fragments of $A\beta_{42}$ in addition to $A\beta_{1\text{-}42.}$ Most of the data suggest that these methods actually measure monomeric free $A\beta_{42}$, but correlate well with a SRM (selected reaction monitoring) based mass spectrometry method for total $A\beta_{42}$[288].The tau protein exists in several isoforms and can be phosphorylated on different residues. The most common ELISA test for t-tau identifies all the isoforms, regardless of their phosphorylation status[289].

The most widespread ELISA tests for p-tau measure phosphorylated tau at residues 181 or 231[290,291] and the diagnostic performance between the different methods is similar. A multiparameter method for the simultaneous measurement of $A\beta_{42}$, p-tau and t-tau has also been developed[292].

1.9 Treatment

To date there is no etiological therapy for AD treatment: the available treatments offer relative symptomatic benefits and remain palliative.

1.9.1 Conventional therapies

1.9.1.1 Cholinergic therapy

AD is above all a functional synaptic damage, more than a structural one; in particular the Acetylcholine (Ach) is the neurotransmitter that turns out to be more reduced in the AD. During cholinergic synaptic neurotransmission, Ach is released from the presynapesis in the synaptic wall and binds to post-synaptic muscarinic or nicotinic receptors. Cholinergic transmission is blocked by acetylcholinesterase (AchE), which catalyses the hydrolysis of Ach. The enzyme choline-acetyltransferase (CAT) catalyzes the synthesis of acetylcholine from precursors, choline and acetylcoenzyme A and, therefore, the loss of activity on the part of CAT would reflect the loss of functional transmission of the mediated nerve transmission of Ach. At the moment, different therapeutic strategies have been implemented based on the different stages of cholinergic transmission, to improve function. Among these there are:

a) AchE inhibitors (AchEi), which increase the concentration of Ach in the intersynaptic space;
b) Ach precursors such as choline;
c) Ach promotors, which promote the release of Ach from the presynapse;
d) Muscarinic agonists, which activate the muscarinic receptor;
e) Nicotinic agonists, which activate the nicotinic receptor.

The effects of AchEi can be classified into:

- Symptomatic clinical effects (related to the blocking of AchE);
- Effects on APP metabolism through muscarinic receptors (which can stimulate a non-amyloidogenic response, stimulating the action of α-secretase, an enzyme responsible for APP metabolism in a physiological way);
- Nicotinic receptor-mediated neuroprotection effects.

Tacrine, a reversible AchE-i, was the first molecule approved for trade in 1983; however it had some disadvantages such as the daily multi-administration (four times a day), a dangerous but reversible liver toxicity (up to 50% of

patients), and poorly tolerated side effects of cholinergic origin (nausea, vomiting and diarrhea). It has been replaced, btween 1996 and 2001, by other AchE-i with similar effectiveness but different mechanisms of action. First of all the *Donepezil*, a reversible AchE-i, which has the advantage of a daily single administration, excellent bioavailability, and the absence of hepatotoxic effects. *Rivastigmine* is a pseudo-reversible AchE-i that has the advantage to be available also as transdermal formulation (reversible and non-competitive antagonist of acetylcholinesterase, butyrylcholinesterase inhibitor), while *Galantamine* is a nicotinic agonist (specifically it is a tertiary alkaloid, selective and competitive inhibitor of acetylcholinesterase, which presents a modest activity also on presynaptic nicotinic receptors). These drugs do not provide a cure for AD, but stabilize or slow down some symptoms for a limited period of time, generally from 6 to 18 months. The use of AchE-i in clinical practice is limited mainly due to its side effects on the peripheral organs (abdominal cramps, nausea, vomiting, diarrhea, anorrectia, heart rhythm disorder), since Ach is the main neurotransmitter in the body. Most clinical trials and revisions conducted thus far have not shown significant differences on the cognitive effects of these drugs[293-297]. On the cotrary, differences were reported in the incidence of side effects, predominantly of gastrointestinal origin (nausea, vomiting, diarrhea), generally minor for donepezil compared to galantamine and rivastigmine[298-300]. The tolerability of the treatment is particularly important in a condition such as AD, often being elderly patients with many comorbidities and in multi-drug treatment.

1.9.1.2 Glutammatergic theory

Glutamate is the main excitatory neurotransmitter; it is active in about 1/3 of the CNS synapses, and is present in regions associated with memory and cognitive functions (such as the hippocampus). Glutamate-induced neuronal hyper-excitability causes calcium accumulation in nerve cells, resulting in neuronal death. *Mementin* is a non-competitive NMDA receptor antagonist without interfering with the phasic activity of the receptor itself, necessary for learning and memorizing, it performs neuroprotection (possible slowing of the disease) and exerts positive effects on synaptic plasticity, on memory and learning functions (improvement or stabilization of the clinical picture). In particular, it

performs a voltage-dependent (it allows the physiological activation of NMDA receptors during the formation of the memory trace and blocks the pathological activation of the same receptor) and an use-dependent action (the antagonist inhibitor action is greater in the sites with receptor response[301,302]). Today Memantine is approved in patients with moderate AD intolerant or with contraindications for AchE-i and in those with severe-moderate AD (to date the only drug with this indication)[303-305]. Co-administration of Memantine-AchE-i is tolerated and safe, but not "more effective"[306,307]. Overall, Memantine is a well tolerated drug that can temporarily improve the clinical picture of dementia, slow down cognitive decline and loss of functional autonomy, and reduce behavioral disorders. However, there is no current evidence that it changes the natural history of the disease. Its adverse events, generally infrequent and moderate, are hallucinations, confusion, headache and fatigue.

1.9.2 Other therapeutic strategies

Neuropathological studies have shown that the brains of AD patients have high concentrations of inflammatory molecules, cytokines and complement proteins. For this reason, many studies have been carried out to evaluate a possible association between the use of NSAIDs or corticosteroids and the appearance of disease. The results are nonetheless contrasting[308-314].

Oxidative stress has often been implicated in the pathogenesis of neurodegenerative diseases. Therefore, drugs with antioxidant properties have been evaluated as treatments for AD. Vitamin E has shown the ability to slow the clinical progression from moderate to severe AD, although cognitive benefits have not been shown[315].Initially, epidemiological studies had suggested a reduction in the risk of AD in patients taking statins[316,317], as it was shown that they could inhibit the formation of Aβ in vitro. However, a more recent meta-analysis has supported these benefits[318], and the same is emerging from multicentric clinical trials[319].

In the 1990s, many population studies and control cases were established to correlate the use of estrogens and the risk of AD. In fact, basic evidence supported the idea that estrogens could be neuroprotective, increasing neuronal growth, connection and survival, stimulating the production and sensitivity of the

NFG. Anyway, results obtained from clinical trials that used conjugated equine estrogens in women with moderate early-stage AD failed to demonstrate improvements in the cognitive framework or a slowing of dementia[320-322]. Even in women over the age of 65, the use of estrogen or estrogen/progesterone increased the risk of converting MCI into dementia[323,324]. Currently it is therefore difficult to imagine any clinical benefit from the use of estrogens.

Docosahexaenoic acid (DHA) is an omega 3 polyunsaturated fatty acid found in fish and some marine algae. It is a component of the plasma membranes of synapses, and, in animal studies, has been shown to perform many functions in the brain, affecting signal transduction mechanisms, neuroprotection and regulation of gene expression. Individuals with AD have lower levels of DHA; in patients with initial forms of the disease, the omega 3 fatty acids slow down the cognitive decay, while no effect was shown in the moderate forms[325].

Ginkgo biloba, a Chinese medicinal herb used for its healthful properties, including memory effects, was tested to assess in elderly subjects a possible reduction in incidence of all forms of dementia. In light of the fact that there is currently no evidence on its cognitive benefits[326], we must consider its dangerous side effects, such as an increased risk of bleeding. Therefore, at present research on ginkgo biloba is "inconsistent and groundless".

The Framingham and other study showed that hyperhomocysteinemia is a risk factor for cardio- and cerebro-vascular disease, as well as for AD[327]. Correction with folate and B12 vitamins, although it traces homocysteine levels, does not slow cognitive decline[328,329].

1.9.3 New therapeutic strategies

The molecules currently being tested intervene to reduce the load on amyloid deposits. In particular, the mechanisms of action of these molecules are 1) the modulation of the formulation of Aβ; 2) the blockage of Aβ aggregation in oligomers and plaques; 3) the increase in Aβ elimination by immunotherapy. Several drugs have been tested as a possible cure for AD, such as latrepiridine (an N-methyl-d-aspartate antagonist), neuronal growth factors, resveratrol (an antioxidant present in grape skins), blue methylene (which blocks the aggregation of the tau protein), agonists of the serotonergic receptor 5-HT4, and β and ϒ

secretase inhibitors.

Studies on β secretase inhibitors have shown conflicting results with preclinical studies; those on asi secretase inhibitors (LY430139 dihydrate) showed a slight reduction of the $A\beta_{1-40}$ fragment (clinical phase II study). However, it showed a biphasic response (an initial reduction in Aβ, followed by a period of increased Aβ) and there were no significant reductions in Aβ in cerebrospinal fluid in treated patients[330]. They have side effects, including gastrointestinal one, and interfere with lymphocyte maturation[331]. Many trials have focused on the role of immunotherapy in AD, with the aim of stimulating the immune system to recognize or introduce preformed antibodies to prevent the deposition of Aβ plaques or increase plaque elimination. The immunotherapic treaments are distinguished in:

- Active immunization (as AN-, which stimulates the production of Aβ antibodies, interrupted because it causes meningoencephalitis), and ACC-001;
- Passive immunization: AAB-001 (Bapineuzumab), BIIB037 (Aducanumab), INN (Solanezumab), by directly injecting anti-β-antibodies synthesized in the laboratory.

1.9.3.1.1 Bapinezumab

Bapinezumab is a humanized monoclonal antibody that acts on the nervous system, and is under study as treatment for AD and glaucoma. Specifically, it binds to the Aβ N-terminal regions. However, in 2012 two major studies failed to produce significant cognitive improvements in patients[331,332], despite the reduction of the main AD biomarkers, such as cerebral amyloid plaque and phosphorylated tau protein in cerebrospinal fluid[333]. The most frequent side effect was vasogenic edema, as assessed by brain MRI.

1.9.3.1.2 Aducanumab

It's a human monoclonal antibody that selectively targets Aβ aggregates. In a transgenic model, Aducanumab has proved itself capable of entering the brain and binding parenchymal Aβ, thus reducing soluble and insoluble Aβ in a dose dependent manner (as confirmed by investigations obtained with PET with β-

amyloid tracer). In patients with mild MCI or AD, one year of monthly intravenous infusions of aducanumab reduced brain β-amilode in a dose and time dependent manner[334]. Clinically, this results in an improvement in scores obtained at MMSE and CDR[335]. The drug proved to be safe (when the drug was used at a higher dose the most commonly reported side effects were headache and cerebral edema)[334]. These outcomes justify the further development of aducanumab for the treatment of AD.

1.9.3.1.3 Solanezumab

It's a humanized IgG1 monoclonal antibody, directed against the central domain of Aβ peptide. It recognizes the non-fibrillar fragments of Aβ monomers, reverses the balance between the different species of Aβ and removes the small soluble species of Aβ, toxic to synaptic function. Its final action results in blocking the formation of β-amyloid plaques. Two large studies (EXPEDITION-1 and -2) have shown that solanezumab is effective in slowing dementia in mild forms and is safe[335-336]. The only adverse event with an incidence of at least 1% in both trials, and more common in patients treated with solanezumab, was angina (1.1% versus 0.2%). Vasogenic edema occurred in 11 patients treated with monoclonal antibody and in 5 of those treated with placebo, without statistical significance. EXPEDITION 3 confirmed the efficacy of the solanezumab antibody, which does not block the disease, but slows down the cognitive decline by 34% as extimated at the end of the 18 months of treatment[337]. The effect on functional decline is not a statistically significant datum when compared to the receiving placebo group, however it showed a reduction of 17%[337].

1.9.4 Symptomatic and rehabilitative therapies

They intend to control cognitive and non-cognitive symptoms (depression, anxiety, behavioral and sleep disorders, hallucinations), to optimize the functional level and improve patients' quality of life. Non-pharmacological therapies primarily rely on rehabilitative interventions aimed at changing the social and environmental situations in which the patient lives; for this reason, the involvement of family and competent social and professional structures plays a role of prime importance in allowing the patient to achieve the best possible

quality of life from a physical, functional, social and emotional point of view. The main safeguards are represented by memory training, reminiscence, remotivation and occupational therapies. Different methods of alternative approaches to drugs in AD have been developed, especially in mild to moderate forms, striving for improvement of the patient's quality of life, reducement of disability and slowing down the course of the disease. These interventions act on the cognitive, cognitive-behavioral, relational and emotional sphere. Actually their effectiveness has not yet been scientifically proven[338-340].The most experienced and commonly used ones are the following:

- **Mild to moderate phases**: memory training, memory rehabilitation, Reality Orientation Therapy (ROT). They require the active collaboration of the patient, teaching him strategies to improve his memory and learning skills. External aids can be used, including diaries, calendars, journals and interiors, or mental strategies, such as logical connections. Instead, reality therapy (ROT) is intended to maintain cognitive contact with reality (space, time, person) through continuous stimuli. It distinguishes itself in formal and informal, depending on whether the information is given in specific sessions or as a reinforcement of environmental and relational messages. This technique has achieved a certain scientific validation: there are evidences of short-term benefits, a reasson why patients would need continuous treatments[341,342].
- **Moderate-severe phases:** validation therapy, music therapy, pet therapy. The validation therapy proposes to follow the patient in his world without bringing him back to his individual reality. Today, music therapy is finding more and more applications, and it seems useful to recall memories or life experiences and to reduce stress. Contact with animals is useful for stimulating activities, memories and affective interaction.

Drugs used for the treatment of non-cognitive symptoms are neuroleptics, mainly the new generation ones, for the treatment of behavioral disorders; benzodiazepines for sleep disorders and anxiety states; antidepressants.

The management of complications, finally, provides an adequate

nutritional intake, the prevention of bedsores through frequent change of position, use of anti-decubitus devices, meticulous hygiene of the skin, careful prevention of falls (including elimination of obstacles, safe furnishings, closure of balconies and windows, suppports to facilitate balance and walking).

PART and SNAP

From 10% to 30% of clinically diagnosed people with AD dementia, according to experts, do not show neuropathological alterations of AD at the autopsy [115] and a similar proportion has a normal level of β-amyloid[116] or Aβ42 CSF[117,116]. Thus the multi-domain anamnestic phenotype of dementia is not specific; it may be the product of other diseases as well as AD[118]. In addition, neuropathological alterations of AD are often present without signs or symptoms, especially in older people. In fact, from 30% to 40% of elderly people without cognitive problems, have neuropathological alterations attributable to AD at te autopsy[120,121,122] and a similar proportion has abnormal amyloid biomarkers[119,118, 123,124,125,126]. Defining AD through neuropathological changes regardless of clinical symptoms represents a profound change of thinking[127].

Once agreed that AD should be defined as a biological construct identifiable in vivo through biomarkers, the next logical question is: which biomarker signature or profile(s) defines AD? Several possible biomarker profiles were considered, the first to become abnormal in carriers of deterministic AD mutations are those for β-amyloid[128,129,130].

These data suggest a primary causal role for β-amyloid in the pathogenesis of AD; while β-amyloidosis alone is not sufficient to cause directly cognitive impairment, it may instead be sufficient to induce pathological changes downstream (namely tauopathy and neurodegeneration) which eventually lead to cognitive impairment. These results are also supported by clinical-pathological studies[31,132]. Consequently, a widespread opinion is that amyloid biomarkers represent the first evidence of neuropathological alterations of AD currently detectable in vivo. However, to satisfy the neuropathological criteria for AD[133,134], either β-amyloid deposits and those coupled with helical filaments (PHF) - which

suggest the presence of abnormalities both in β-amyloid and tau biomarkers - are required.

The level of cognitive decline is significantly higher for patients with cognitive disabilities and for individuals who have alterations of both an amyloid biomarker and a second type of biomarker that could be CSF tau (T-tau or P-tau), atrophy or hypo metabolism, when compared with individuals who have neither of the two abnormalities of these biomarkers[147-152].

Individuals with imaging/biomarker evidence of Alzheimer's disease-like neurodegeneration (AD) without β-amyloidosis have been labeled as "suspected non-Alzheimer's pathophysiology" (SNAP)[153-164]. Crary et al.[165] suggest the creation of an additional neurodegenerative disease called PART ('primary age-associated tauopathy') which describes cases with tau pathology in the entorhinal cortex and in the hippocampus (tau pathology in ECH) without deposits of Aβ (tau + / Aβ-) or with minimal deposits of Aβ. The amount of Aβ deposits compatible with PART and the techniques used to detect them is still to be determined. To all intents and purposes, PART is part of the SNAP subgroup. The tau pathology is topographically limited and "age-related" but not linked to AD. These cases are characterized by a low NFT stage (I-III or IV) with little or no deposition of Aβ (Aβ phase from 0 to 2), tau / Aβ I / 0, tau / Aβ II / 0, etc., with the first score here indicating the NFT stage and the second one the Aβ phase where tau / Aβ is used as an abbreviation for the tau / Aβ pathology deposits.

The main controversy in the literature is whether PART should be considered a non-AD correlated entity but vice versa linked to age or if it should be considered as part of the pathological spectrum of AD. Specifically, the same controversy exists in literature about SNAP imaging/biomarker. Currently there are two major hypotheses for the nosological integration of these cases: one favors unity, that is a continuum from tau + / Aβ- to tau + / Aβ +; the other privileges the concept of PART as a taupathy associated with age (tau + / Aβ-) which is a totally different process from AD (tau + / Aβ +). This duality of processes is thus divided into two opposing sides, AD vs 'aging'[165].

1.10 PART as age-associated taupatia: a different entity from AD

According to the PART as a separate entity hypothesis, cases with tau+/Aβ- differ from AD and would not have reached higher NFT stages than IV and Aβ phases above 2, even if they had lived longer. According to the same hypothesis, tau pathology is associated with aging, while the deposition of Aβ is linked to AD, with the inference that older people should be spared by the latter and influenced instead by the first process.

According to the PART hypothesis, the tau pathology in the ECH, if not accompanied by the deposition of Aβ, will not progress (or is less likely to progress). Very old people. They should be included in these studies to cope with the long duration, and perhaps irregular progression, of tau and Aβ pathologies. A distinction should be made between the age-related increase of the local density of lesions and the increase of the number of affected regions. The prognostic value of the ECH tau pathology quantified through the aging spectrum it's a a topic that should not be ignored.

PART is believed to be common in middle-aged and elderly subjects. SNAP is also common in people over the age of 65. Of 1,425 cognitively normal subjects reported from seven different centers, 315 (22%) were classified as SNAP[166-171] [51, 52, 53, 54, 55, 56]. Of 277 subjects with MCI reported from 4 different studies, 68 (25%) were classified as SNAP [171-173] [56, 57, 58]. Because of the large number of subjects included in these studies (especially cognitively normal ones) the estimated frequencies for SNAP population are probably reliable. In support of PART as an entity distinct from the CEO, there are two key points: first, the rate of Aβ accumulation is not influenced by neurodegeneration and therefore can be a biologically independent process. The rates of β-amyloid accumulation (Aβ) on the PET image are not related to hippocampal neurodegeneration; secondly, the pathophysiology of Aβ increases or catalyses neurodegeneration[174].

Further reasons have been put forward concerning the existence of a specific pathology other than AD[175]:

1. The average age at death is generally higher for patients with PART than for those with AD.
2. There is no association between PART and the APOEε4 allele. An association with the tau H1 haplotype was found in the "predominant tangle" forms.
3. The most serious PART disease is associated with a higher age at death and lower scores in cognitive tests.

1.11 PART as a continuum of AD

In the continuum hypothesis, the tau pathology begins in ECH (NFT stage I to III or IV) in the absence or in the presence of minimum levels of Aβ deposits (phase 0 of Aβ); the tau will subsequently be found in the isocortex (stage NFT V and VI) together with the deposits of Aβ (phase 1 of Aβ or higher). In conclusion, both Aβ and tau pathologies are necessary for the diagnosis of AD according to the current criteria[176-177].

However, the neuropathological data presented in this article support the idea that only the tau component may be evident in the initial phase. The deposition of Tau in the ECH is therefore necessary but not sufficient for the development of AD. The correlation is not causal, in fact: there is the hypothesis that Aβ causes or promotes the tau pathology, or that the tau pathology causes or promotes the development of the AD pathology, or maybe there is a third variable (currently unknown) responsible for the synchronous development of the two pathologies.

Therefore in a patient who develops PART and subsequently AD it must be considered that PART is an "age-related" tauopathy and that tau is initially found in ECH (NFT stage I, II or III). So the question is: if at the beginning of AD, Aβ deposits appear in the isocortex (phase 1 of Aβ), does the "age-related" tauopathy initially present in ECH still qualifies as PART as soon as Does Aβ deposition occur, or does it become related to AD as it loses its "pure tauopathy" status? The logic would be to consider that PART remains even when AD starts developing. Should it be considered then that the tau pathology in ECH is mixed (AD- and "age-related"), but that their components cannot be separated because

there is no way, at least currently, to distinguish between the two entities? Or that PART invariably evolves into AD and is therefore a pre-clinical form of AD? PART (tau + and Aβ-) is equated to SNAP (brain injury +/Aβ- biomarkers) as discussed in other studies[178] .Therefore the previous observations also apply to SNAP. However, there may be a way to reconcile these opposing perspectives on PART and SNAP. A series of recent publications in the imaging / biomarker literature proposed the following gradual scenario as a common pathological sequence and biomarker in late-onset AD[179-182]. This proposed pathological sequence is actually based on previous autoptic publications[183-185].

1. Essentially every individual in the population develops PART at some point in his life. This usually occurs before a significant deposition of fibrillar amyloid. However, PART by itself produces no symptoms or at most an MCI (mild cognitive impairment).
2. Independently of PART, β-amyloidosis develops in neocortical areas[184,185].
3. At some point, considerevely differently from person to person and through still undetermined signaling mechanisms, β-amyloid begins to induce the spread of tauopathy from the neocortical to the temporal medial association areas.
4. The severe clinical symptoms are due to the direct involvement of neocortical areas by accelerated and expanding tauopathy, and not linked to the β-amyloid deposition.

In this model of late-onset AD[179,180,181,182,183,184] the role of β-amyloid is to induce the propagation of tauopathy, rather than starting the first deposition of tau in the brain (as probably occurs in the case of genetically determined AD[188]. If the continuum hypothesis is correct, the risk of developing AD will be significantly increased in cases of ECH tau pathology and the ECH tau pathology would always precede the full-blown AD in a period of time that has yet to be determined[189].

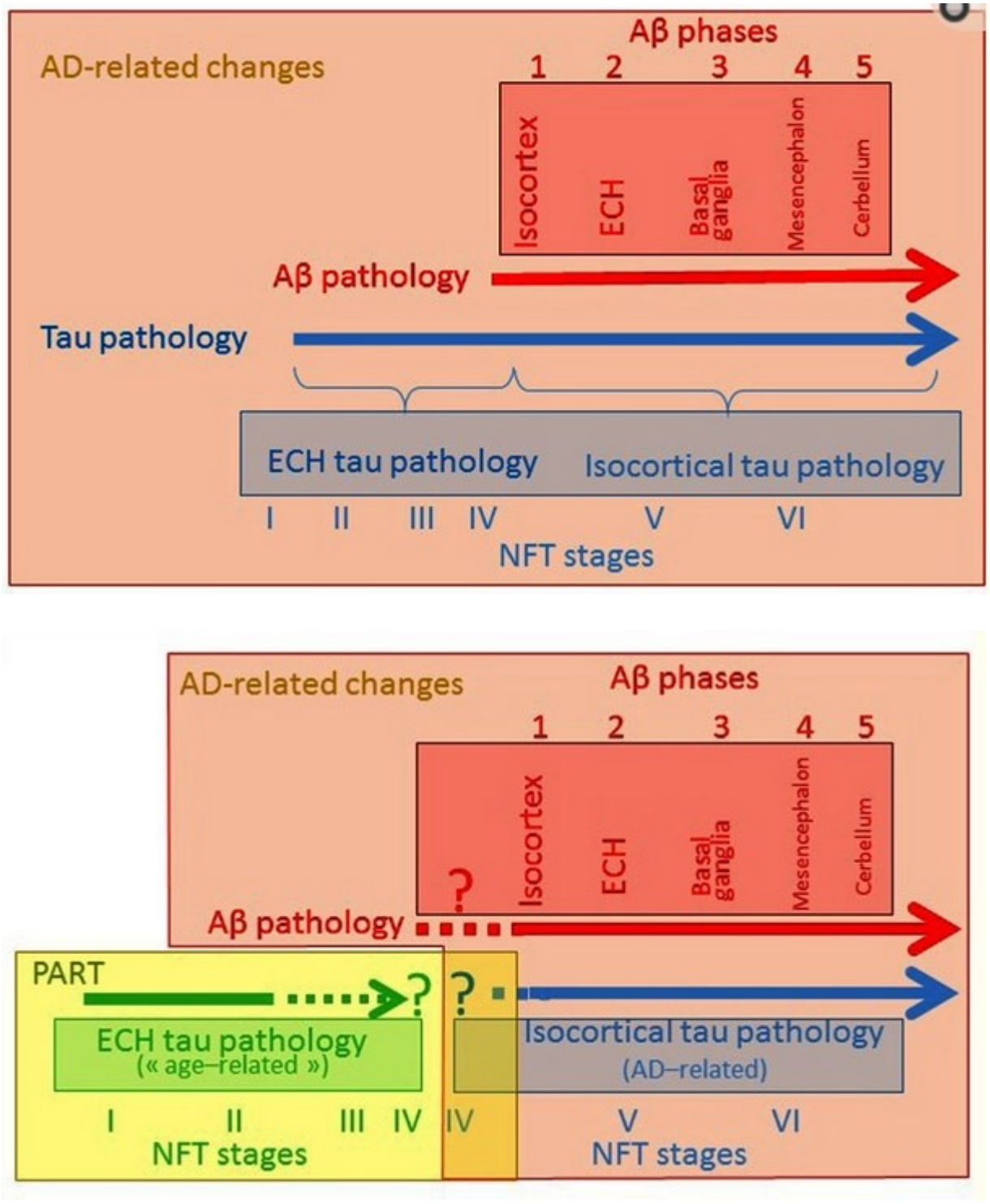

Fig.12 Relationship between tau-related pathology and Aβ deposition.

In relation to this it's necessary to ask the following questions: can the deposition of Aβ precede the tau-related pathology? Can PART manifest itself in association with phase 1 or 2? Where and how does PART end?

In conclusion, it is still to understand why in PART, tau remains confined to the ECH; given that, by definition, PART has high levels of tau but low / absent amyloid levels, it is possible that it is precisely the latter's presence that causes it to progress in AD thus inducing the tau to spread also in other brain segments. As for tau pathologies (SNAP and PART), they follow a hierarchical distribution, which occurs in an ordered series of regions: it can be found only in the entorhinal cortex, in the entorhinal cortex and in the hippocampus, or in the entorhinal cortex, in hippocampus and neocortex[190]. Aβ deposits can be found only in the neocortex, in the neocortex and in the hippocampus, or, moreover, in the basal ganglia, in the midbrain and in the cerebellum[191]. The number of areas involved in the tau and Aβ pathology increases continuously in a defined sequence. It also implies a progression independent from the lesion density. This is the basis for the identification of the stage (NFT) for the evaluation of tau pathology[190-192] and Aβ phases (evaluation of Aβ deposits)[191].

Chapter Two

Objectives

Evaluation on how the value of beta-amyloid correlates in CSF and PET (cerebral), in order to establish a different biomarker profile between Alzheimer continuum and SNAP in light of the new NIA-AA guidelines.

Materials and methods

1.12 Study population

40 patients accurring to the Nervous System Diseases Clinic of the OORR in Foggia were recruited from September 2017 to June 2018. In day-hospital regimen, patients underwent blood tests (including blood counts, liver, kidney, thyroid, vitamin B12, folate tests and syphilis tests), detailed neuropsychological evaluation and conventional brain MRI (including T2-, T1-, FLAIR and diffusion-weighted images) and VNP. If early-stage AD was diagnosed, in-patient rachicentesis and cerebral amyloid PET were carried out.

Depending of the rachicentesis, patients were divided into 2 groups according to the CSF values of IATI and 181p-Tau

1) AD: 181p-Tau > 61 pg/ml and IATI <1.2 (CSF+)
2) SNAP: 181p-Tau <61 pg / ml (CSF-)

1.13 Neuropsychological evaluation

Patients underwent neuropsychological evaluation, including Mini Mental State Examination (MMSE), long-term verbal memory test (prose memory), short-term verbal and spatial memory test (Digit Span, forward, backword span and Corsi test), visuospatial tests (test of the drawing of the clock [CDT] and tests to evaluate executive abilities (FAB, fluency by letter and by category) and attention (Attention matrices). Mood disorders were assessed using the Geriatric Depression Scale [GDS]. ADL (Activities of Daily Living) and IADL (Instrumental Activities of Daily Living) were used to assess patients'autonomy.

1.14 Informed consent

The study is approved by the Ethics Committee of OORR Foggia and all subjects provide informed consent in written form to participate in the study, before taking part in it.

1.15 Cerebrospinal fluid examination

Lumbar puncture was performed in all recruited patients. The liquor obtained was collected in 12 ml polypropylene tubes and centrifuged at 2171 rpm for 10 minutes (3400 rpm) within 2 hours from collection. A small amount of cerebrospinal fluid was used for routine analyzes (total cell count, total protein and glucose levels), and bacteriological and microbiological tests. The CSF was then temporarily aliquotated in polypropylene tubes and stored at -22° to quantify, within a maximum of 1 month from collection, the following biomarkers: $A\beta_{1-42}$, 181 p-tau and total tau through the innotest ELISA (Innogenetics). Finally, the IATI index (Innotest Amyloid Tau Index) was calculated.

1.16 Cerebral beta-amyloid PET

The sixteen patients underwent an amyloid-PET test. In 12-hours fasting condition, 185 MBq of flutemetamol (18F) were administered intravenously as a bolus within about 40 seconds. The injection volume being between 1 ml and 10 ml. The activity of flutemetamol (18F) was measured with a dose calibrator immediately prior to injection. Patient was supine so that his brain (including the cerebellum) was within a single field of view. Brain scans were acquired about 90 minutes after the radiopharmaceutical injection. PET images were acquired through a 128-layer *Discovery PET / CT 600* (*GE HealtHSare*) scanner, with a temporal CT resolution of 0.25 sec and a spatial PET resolution of 2.14 mm (*FWHM)*. The images were reconstructed using a filtered method of three-dimensional rear projection for a total duration of the examination of about 20 minutes. Images were processed with 3DSSP

(http://128.208.140.75/~Download/).Three groups of patients emerged from the correlation between PET-amyloid and CSF images:

1) AD: group positive for symptoms (symptoms +), 181p-Tau >61 pg/ml and IATI <1.2 (CSF +), amyloid-PET images positive for β-amyloid uptake (PET +).
2) AP: group positive for symptoms (symptoms +), 181p-Tau <61 pg/ml and IATI >1.2 (CSF-) and amyloid-PET images negative for β-amyloid uptake (PET +).
3) SNAP: group positive for symptoms (symptoms +), 181p-Tau <61 pg / ml and IATI >1.2 (CSF-) and amyloid-PET images negative for β-amyloid uptake (PET-).

1.17 Statistical analysis of amyloid-pet images

Statistical analyzes were performed at the Department of Hospital Neurology of Foggia. The PET-amyloid interfiles were converted into NIFTI format and pre-processed according to the PET protocol implementing in SPM12 (www.fil.ion.ucl-c.uk/spm/). In short, the images were first aligned, normalized and then smoothate using a 12 mm FWHM Gaussian kernel. The interaction between CSF and PET was studied in the three cohorts of patients through the statistical analysis method of varianca "ANOVA" which allowed to compare the data relating to these three groups. The statistical tests were all performed with a *whole brain* approach and the results considered statistically significant for a corrected pFWE value <0.05% on the cluster level

Results

The three groups AD, AP and SNAP, classified according to CSF profile and PET imaging, were homogeneous for age, schooling and scores at neuropsychological tests (MMSE, ADL and IADL) but were not homogeneous for CSF values of β-amyloid.

	AD	AP	SNAP	VARIANZA TRA I GRUPPI AD,AP,SNAP	
	n=18	n=10	n=12	Fstat	P value
Age (years)	66.4(8)	70(4.4)	65.5(8.7)	0.7	N.S.
Sex(female/male)	2/5	3/5	2/4	7.7	N.S.
MMSE score	19.5 (5.5)	18.6(5.2)	22.8(4.5)	2.5	N.S.
ADL score	6(0)	5.5(0.8)	5(1.7)	1,75	N.S.
IADL score	4,4(1.9)	3.17(2.2)	3.7(4.0)	0.4	N.S.
Years of formaleducation	8(3.6)	6.7(3.3)	8.7(3.6)	0.5	N.S.
Aβ1-42value	361.7(181.4)	435.9(201.4)	806.5(370.5)	6.97	p<0.05
	Mean(SD)				

Tab.2. Clinical and demographic characteristics of AD, AP and SNAP patients. The results were considered statistically significant for a value of p <0.05 and critical F.

Aβ1-42		Bonferroni-Holm test
AD	AP	0.05
AP	SNAP	0.0025
AD	SNAP	0.017

Tab. 3. Different patterns of β-amyloid accumulation. The analyzes showed a different and significant (p<0.05) pattern of β-amyloid accumulation in the three groups in the table. The major and significant differences in terms of β-amyloid accumulation occur in the AP *versus* SNAP group (p = 0.0025), AD *versus* SNAP (p = 0.017), and AD *versus* AP (p = 0.05) respectively.

1.18 Brain MRI

Brain magnetic resonance images were examined by an expert radiologist, who confirmed the expected picture of brain atrophy (with bilateral temporo-parieto distribution) in all patients with probable AD.

1.19 Cerebral beta-amyloid PET

Among the three groups (AD, AP and SNAP) significant differences emerged in the accumulation patterns of cerebral β-amyloid

1) COMPARISON AD *VERSUS* AP: significant accumulation of cerebral β-amyloid at hippocampal level in AD compared to AP ($p < 0.05$ at cluster level). (Fig.13)

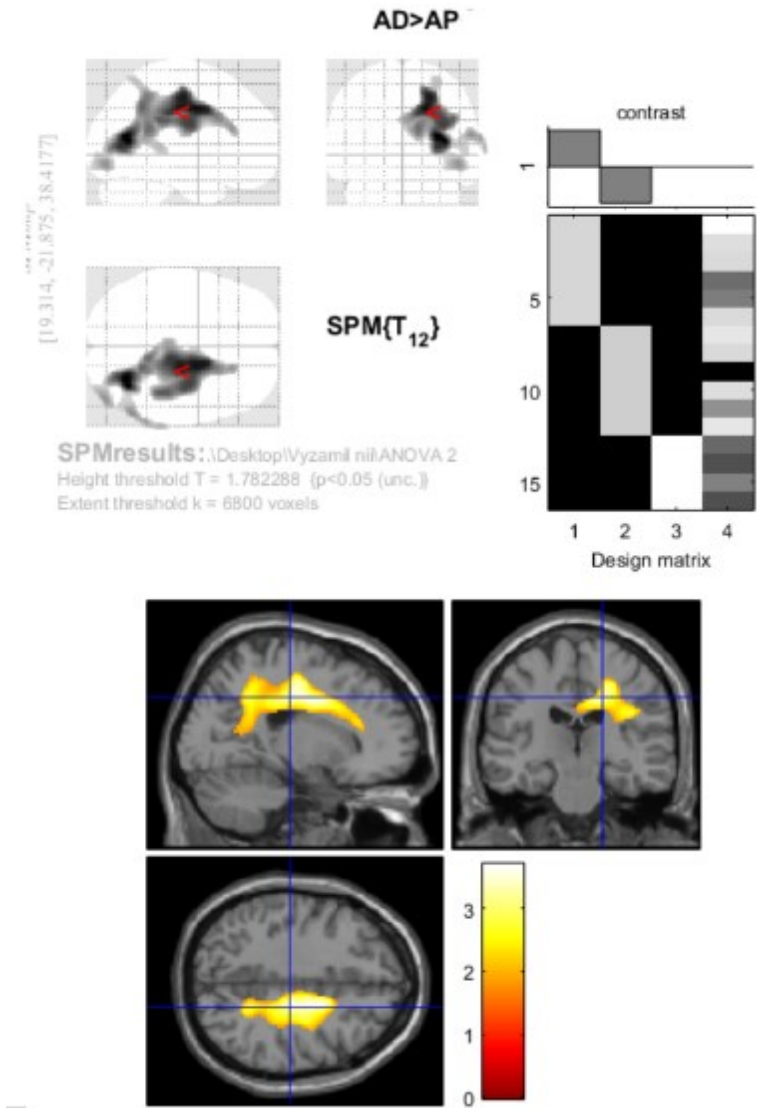

Fig. 13. Brain β-amyloid distribution pattern between AD and AP.

The relevant areas in terms of difference in distribution of cerebral β-amyloid are shown in yellow.

2) **COMPARISON AP *VERSUS* AD:** significant accumulation of cerebral β-amyloid at the level of the frontal regions in the AP group compared to AD ($p < 0.05$ at the cluster level). (Fig. 14)

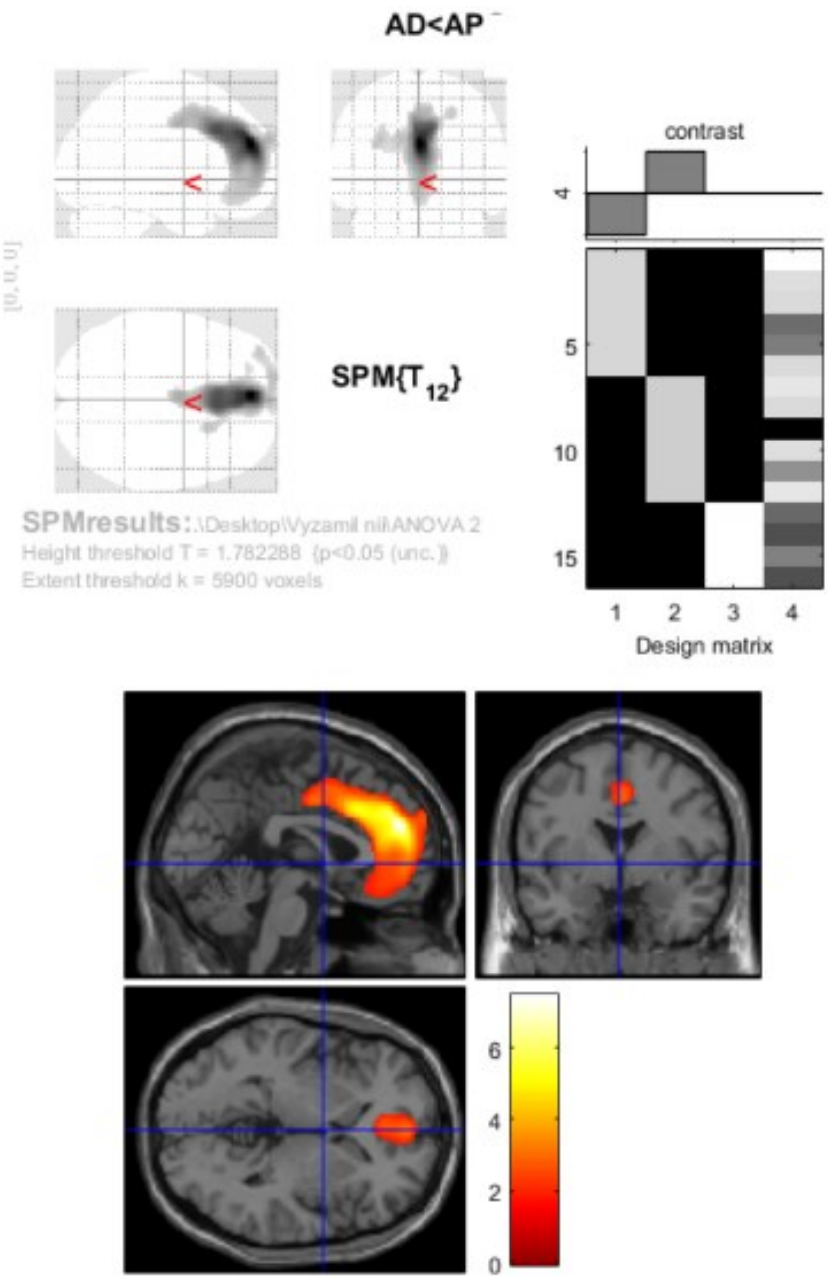

Fig. 14.Brain β-amyloid distribution pattern between AP and AD

The relevant areas in terms of difference in distribution of cerebral β-amyloid are shown in yellow.

3) **COMPARISON AD *VERSUS* SNAP:** significant accumulation of cerebral β-amyloid in all brain areas in the AD group compared to SNAP ($p < 0.05$ at the cluster level). (Fig. 15)

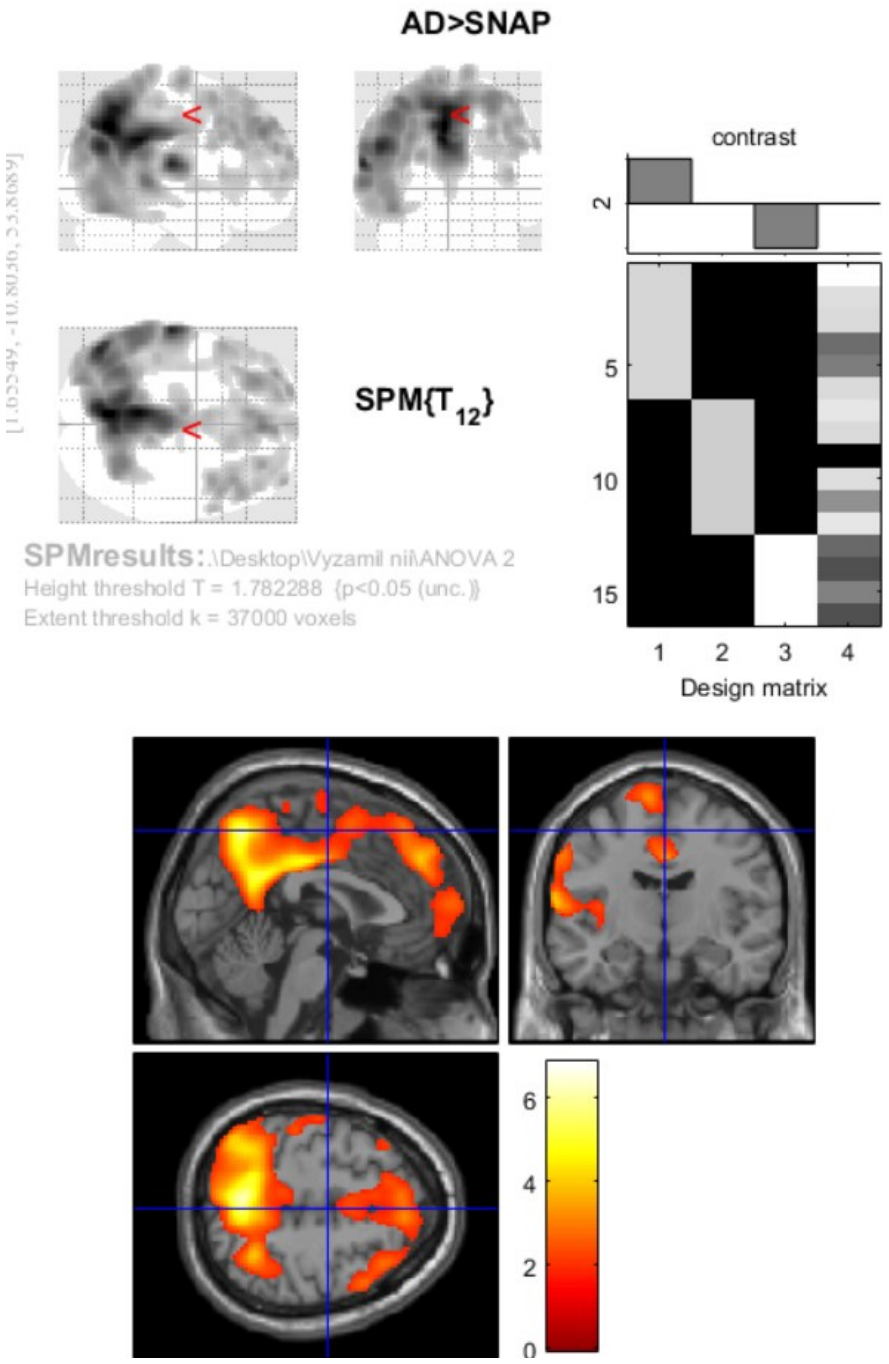

Fig. 15.Brain β-amyloid distribution pattern between AD and SNAP

The relevant areas in terms of difference in distribution of cerebral β-amyloid are shown in yellow.

4) **COMPARISON AD *VERSUS* SNAP:** significant accumulation of cerebral β-amyloid in all brain areas in the AD group compared to SNAP ($p < 0.05$ at the cluster level). (Fig. 16)

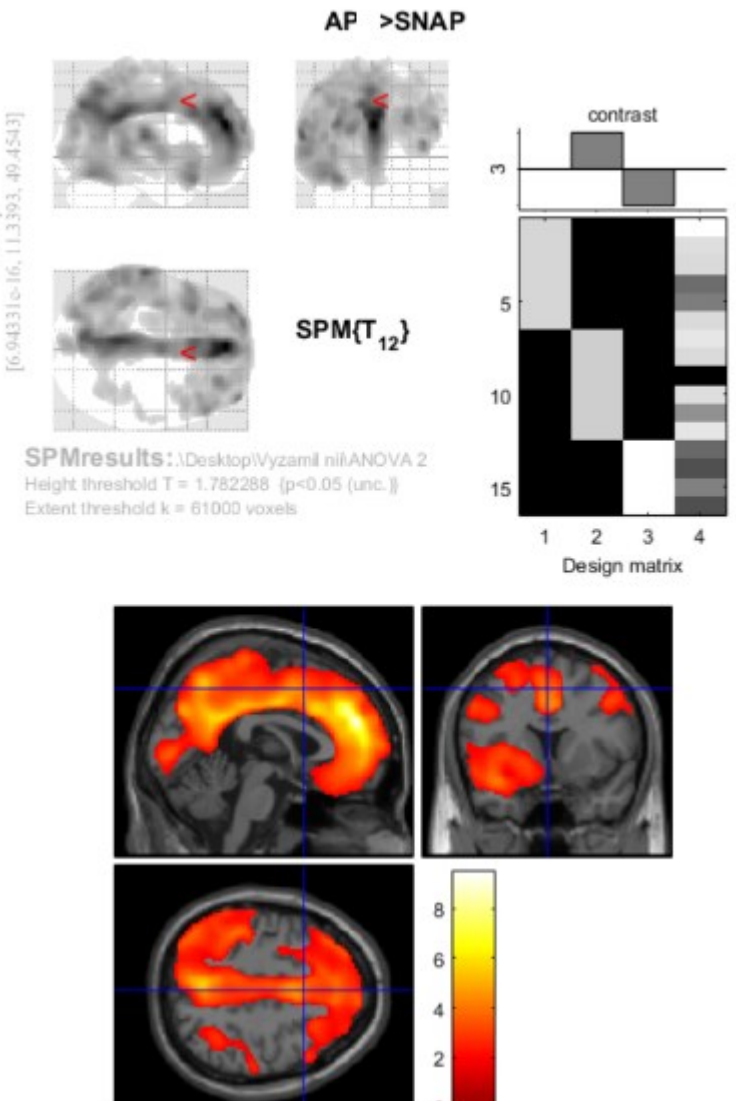

Fig 16. Brain β-amyloid distribution pattern between AD and SNAP.

The relevant areas in terms of difference in distribution of cerebral β-amyloid are shown in yellow.

DISCUSSION

According to new diagnostic criteria[114], the term AD refers to a set of neuropathological changes that can be evaluated in vivo through CSF and instrumental tests (imaging and amyloid PET), rather than specific clinical symptoms[114]. It is now widely accepted that CSF Aβ42 (or preferably the Aβ42/40 ratio) is a valid indicator of alteration of the pathophysiological status associated with fibrillar deposits of cerebral β-amyloid[142]. Our study has thus analyzed the values of CSF biomarkers in the 16 patients with clinical diagnosis of AD. Two groups emerged: the first one with symptoms+/CSF+ and the second with symptoms+/CSF-. At this point we asked ourselves about the nosological entity of the second group (symptoms+/CSF-). Comparative studies between imaging and post-mortem findings have established that amyloid PET images are a valid in vivo surrogate for the deposition of fibrillar β-amyloid (in the cerebral parenchyma or in the walls of blood vessels)[353-360]; therefore, we put the patients through the PET-amyloid imaging study. The second group was so dichotomically divided into a first group characterized by symptoms+/CSF-/PET + and a second group characterized by symptoms+/CSF- /PET-. Schematically, the three groups obtained are:

1) AD Group: CSF+ / PET+
2) "Alzheimer's pathophysiology" (AP) group: CSF- / PET+
3) "Suspected non Alzheimer's pathophysiology" (SNAP) group:CSF- / PET-

Our study then correlated the amyloid PET images of the three groups in order to highlight any differences in cerebral β-amyloid accumulation. The first comparison was conducted between the AD *versus* AP group and a significant accumulation of β-amyloid emerged in the regions of the posterior cingulate gyrus in AD compared to AP. The posterior cingulate gyrus is involved in maintaining spatio-temporal orientation and memory functions, thanks to the connections with

the paraippocampal cortex[401]. Involvement of the posterior cingulate gyrus is characteristic of patients with a typical clinical presentation of AD[402].

This oitcome is also consistent with the typical Aβ cerebral distribution in the AD (Braak and Braak stages)[105]and actually emphasizes the possibility of deposits in a not-typical location in AP. Therefore, if AP represents a continnuum with AD[114], but the distribution of cerebral β-amyloid does not occur in the typical sites of its accumulation, a different etiopathogenetic mechanism is hypothesized from the AD "typical" one[361]. In support of this hypothesis, the results of the second comparison conducted between AP *versus* AD group, from which a pattern of regional accumulation of cerebral β-amyloid in the AP frontal lobe regions as against AD emerged. Based on this evidence, we hypothesized that AP represents a clinical variant of the AD pathology defined in the literature as "frontal variant of AD"[361]. Several studies have found that in the frontal variant of AD, the NFT (neuro-fibrillary tangle) load in the frontalcortex is about 10 times higher[361] than in the typical AD group. On the other hand, patients with typical AD showed a greater accumulation of NFT in the entorhinal cortex, cingulate gyrus and temporal cortex[361]. Both data and evidence are consistent with the results of our study.

Starting from the analysis of the neuropsychological tests carried out in AP patients (Behavioral Assessment of the Dysexecutive Syndrome, BADS) behavioral and language alterations emerged at the disease onset, in addition to the memory impairment which is a pathognomonic sign of the typical AD. The typical AD refers to a pattern characterized by an early episodic memory loss followed by various combinations of deficits including attentional-executive deficit, language and visuospatial capacity deficits, which reflect the spread of the disease from the medial temporal lobe to other neocortical areas[362-365]. In contrast to this typical profile, the focal cortical variants of AD[366] exhibit an atypical symptomatology. The frontal variant of AD, in fact, is characterized by severe impairment in the tests investigating the functioning of the frontal lobe in the mild stages of dementia[367,368] which include executive dysfunctions[369,370] impairment in design skills, behavioral anomalies, impulsiveness, inattention to details, inability to plan and language deficit[371]. Despite the serious alterations at the tests for the

functioning of the frontal lobe, the performance of neuropsychological tests were similar to the typical AD group. This suggests that a severe frontal deficiency is the main neuropsychological feature of an otherwise typical AD profile, therefore, although the group with frontal AD variant exhibits some clinical features similar to other dementias involving the frontal lobe (FTD), the clinical profile of the frontal variant of the AD is more similar to that of the AD[361].

Therefore assumed that AP represents the frontal variant of the AD, what can be the pathogenetic explanation that justifies the atypical clinical presentation and the selective accumulation of Aβ at the frontal level? Several studies suggest that the deposition of fibrillar Aβ explains at most a small part of the clinical-anatomical heterogeneity of Alzheimer's disease[361]. Indeed, an increase in tau fibril tangles but not in amyloid plaques was observed in the frontal variant of AD. This evidence suggests that tau tangles, rather than amyloid plaques, contribute to the atypical clinical presentation of the frontal AD group[372-375].

It is now widely accepted that in AD the neurofibrillary lesions start accumulating in the limbic and temporoparietal regions and only afterwards they would progress towards the frontal and occipital cortex. Thus the frontal lobes would be affected by neurodegenerative lesions typical of AD in a subsequent temporal sequence. This predictable sequence is assumed to occur with a minimum individual variation[105]. It is therefore possible that in the AD variants there is a focal deficit that is indicative of a selective, early and prominent vulnerability of some brain regions which generally, as mentioned, will normally be involved in the AD pathology in a subsequent time sequence. This vulnerability would be caused by the primary tau deposition in the frontal region[376-380]. On the other hand, the frontal variant of AD is characterized by a pathological process that does not seem to remain limited to the frontal lobes for a long time[366], which is consistent with the NIA-AA definition of AP in continuum with AD[114].

In summary, there are 2 possibilities: (I) the NFT pathology has an earlier onset in patients with frontal AD or (2) NFT pathology accumulated at a faster rate in patients with frontal AD. Therefore in the AD, the initial event of the

cascade that induces the pathology is the aggregation of the amyloid-β, but in the frontal variant, is the neurofibrillary pathology that leads neurodegeneration[361].

Studies have shown that amyloid-β deposition and local tau aggregation in mouse models correlate with neuronal activity[361,381] and that the accumulation of amyloid-β in humans occurs in highly interconnected cortical areas[382], also in preclinical stages[383,384]. It is interesting to note that recent in vitro studies [385,386,387], as well as in vivo results on transgenic mice[389] have found that misfolding and tau aggregation could spread through synaptic connections, thus leading to disease progression within specific neural networks interconnected between their.

These observations suggest that amyloid-β aggregation would be driven by the total flux of neuronal activity while tau aggregation would depend on trans-neuronal diffusion, producing neurodegeneration models that coincide with specific functional networks that eventually lead to specific clinical phenotypes (AD variants)[361]. Validation studies of PET tau ligands are underway: this method could confirm our hypotheses.

A better understanding of the factors that drive the heterogeneity of these clinical phenotypes can provide important insights into the mechanisms of Alzheimer's disease and have direct implications on the diagnosis and management of patients with emerging disease-specific therapies[366].

Finally, in our study, the third and fourth comparisons were conducted respectively between the AD and SNAP groups and the AP and SNAP groups. Both groups showed a significant pattern of accumulation of cerebral β-amyloid almost widespread in all brain areas. This result is not surprising, since that SNAP is a syndrome defined by both normal levels of amyloid biomarkers (CSF- / PET-) and MRI or FDG-PET neurodegeneration patterns[389].

From 10% to 30% of clinically diagnosed people with AD dementia, according to experts, do not show neuropathological alterations of AD at the autopsy [115] and a similar proportion has a normal level of β-amyloid[116] or CSF Aβ42 [205,214]. Thus the multi-domain anamnestic phenotype of dementia is not specific; it may be the product of other diseases as well as AD[116].

To date, SNAP remains a not yet well-defined nosological entity. The clinical diagnosis of AD is often "incorrect" but there are significant differences

with regard to clinical progress, genetic susceptibility and progression of the pathology, which have crucial implications for a precise and correct diagnosis, for clinical management and effectiveness of clinical trials on drugs[399].

SNAP is a very frequent condition in clinically normal subjects > 65 years and appears to be age-related. A study found that the frequency of SNAP was 0% in the age group between 50-60 years while it reached 24% around the age of 89[400]. The frequency of SNAP is therefore not a static, but a dynamic element that increases with age. It was also highlighted that SNAP subjects tend to be older than AD subjects in the preclinical phase[400]. However, literature does not agree with this concept, considering that patients with a genetic predisposition may not show a significant amyloid load on the performed examinations.

There were no differences in the level of education between SNAP groups and those with other biomarker profiles and no racial predilection was related to SNAP even though the few studies that reported the ethnicity of the participants were conducted on Caucasian subjects making it therefore difficult to observe inter-racial differences[396,397].

Furthermore, according to a study the proportion of APOE ε4 in the SNAP group would be much lower than that in individuals with preclinical AD[398].

These observations supported the idea that SNAP is not simply the result of errors in measurement or classificationbut is rather an entity with a strong biological foundation[400].

The main controversy in the literature is whether SNAP is an indipendent pathological entity or can evolve into AD[178]. Some researchers believe that SNAP should be included as an integral part of the AD spectrum; if so, the pathogenetic explanation of the amyloid-centric models of AD and the concept of preclinical AD[395] are wrong and should therefore be reviewed. On the contrary, if SNAP is a different entity from AD, the amyloid-centric models of AD and preclinical AD[395] are completely consistent with current knowledge. In both cases, multiple studies have shown that the pathogenesis of SNAP is linked to the deposition of tau fibrils, which justify cerebral neurodegeneration; it would then be Aβ, even in small quantities, to act as the biological driver of taupathy, and cause the "spread" of tau in a widespread manner throughout the brain[393,394].

The use of Tau PET in vivo will shed light on the etiopathogenesis of SNAP[390-392].

CONCLUSION

This study supports the evidence that a better understanding of the factors that drive the clinical and etiopathogenetic heterogeneity of the three groups (AD, AP, SNAP) may provide direct implications on the correct diagnosis and prognostic accuracy in clinical practice. Furthermore, understanding the different nosological entities in study allows a better stratification of the patients in the future trials and the management of emerging specific therapies for this disease.

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