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Correlation of Alzheimer's Disease Neuropathologic Staging with Amyloid and Tau Scintigraphic Imaging Biomarkers .

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ABSTRACT

PET neuroimaging of amyloid-beta (Aβ) provides an in vivo biomarker for pathologic changes associated with Alzheimer's disease (AD). Aβ targeted agents have been approved by the FDA with additional agents, most notably targeting tau, currently under clinical investigation with one approved by the FDA in May 2020. These agents, along with non-scintigraphic biomarkers from blood and cerebrospinal fluid have provided an opportunity to investigate the pathogenesis, prodromal changes and time course of the disease in living individuals. The current understanding of the neuropathological changes of Alzheimer's Disease (AD) continuum is now proposed to begin up to 25 years prior to the onset of clinical symptomatology. The opportunities afforded by *in-vivo* biomarkers of AD, whether by cerebrospinal fluid examination or PET, have transformed the design of AD therapeutic trials by shifting focus to the preclinical stages of disease. Future disease-modifying therapies, should they be forthcoming, will rely heavily on the use of approved biomarkers or biomarkers currently under investigation to confirm the presence of target pathology. Understanding the progressive neuropathological changes that occur in Alzheimer's disease - and how scintigraphic findings relate to these changes - will serve the interpreting physician to fully appreciate the implications of their findings and provide a basis to interpret their examinations. The recently adopted National Institute of Ageing – Alzheimer's Association (NIA-AA) guidelines define post mortem AD neuropathological changes as a composite score based upon three elements. These elements are the extent of involvement (spread) by cerebral Aβ based on the progression model defined by the Thal Aβ phases, the extent of involvement (spread) by neurofibrillary tangles (composed of hyperphosphorylated tau proteins) based on the progression model defined by Braak and, thirdly, the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) score, which describes the density of neuritic plaques based on certain key locations in the neocortex. This paper will review the three elements that define the NIA-AA scoring system and discuss current evidence regarding how they relate to findings based on Aβ and tau PET scintigraphy.

NOTEWORTHY

- Alzheimer's disease (AD) is characterized by the gradual deposition of Aβ protein plaques and neurofibrillary tangles (tau protein) in characteristic progressive stages
- Post-mortem definition of AD is based on staging of both pathologies as well as neuritic plaque density
- *In-vivo* PET scintigraphy is able to identify, with limitations, both Aβ and NFT pathology
- These new imaging modalities allow for *in-vivo* confirmation of AD

INTRODUCTION

In 1906 Alois Alzheimer described the first documented case of what is now termed Alzheimer's disease (*1*). At age 51, the patient Auguste Dieter began to experience memory loss, hallucinations, psychosocial impairment and disorientation. From 1901 to 1906 she remained hospitalized with a rapidly progressive dementia syndrome, spending her final years in an apathetic bedridden state until her death in 1906. In 1907, Alzheimer published the clinical and neuropathological findings based on her autopsy. Until the 1990s, Alzheimer's disease was clinically differentiated from the more loosely defined 'senile dementia', and was classified as a 'presenile dementia'. Today these diseases are known to represent different variants of the same pathological state. Younger onset cases are frequently caused by highly penetrant genetic mutations while dementia with onset over the age of 65 is typically governed by a combination of modifiable (primarily cardiovascular) and non-modifiable risk factors (age, genetics and sex). It is now known that Auguste Dieter developed her condition due to a mutation in the Presenilin 1 gene. The gene produces the protein presenilin, a component of an enzyme (gamma secretase) that plays a central role in the generation of Aβ peptides from the larger amyloid precursor protein molecule (*2*).

Examining Auguste Dieter's brain Alzheimer described the two hallmark microscopic changes of the condition: extracellular plaques and characteristic intracellular bundles now known as neurofibrillary tangles (NFTs) (*3*). For the following 70 years there was only minimal progress in the understanding of how Aβ plaques and NFTs relate to the pathophysiology of the disease. Medical students in the 1980s were instructed with little information than that from Alois Alzheimer. The extracellular plaques were by then identified as containing a core of misfolded Aβ protein that are frequently but not always surrounded by abnormally configured neuronal processes (neurites). These neuritic plaques, in contrast to pure Aβ plaques, are comprised of a combination of Aβ and NFTs. The NTFs were in turn found to be formed by aggregation of hyperphosphorylated form of tau, a neuronal structural protein in its dephosphorylated form. The rapid progression in the understanding of the pathology of Alzheimer's disease and pre-dementia states such as mild cognitive impairment (MCI (*4*); broadly defined as objective impairment in at least one cognitive domain but preserved independence in functional abilities) over the ensuing 30 years now poses a challenge as these medical graduates are today the senior physicians tasked with performing biomarker scintigraphy of AD. The present paper aims

to address this potential gap in knowledge by reviewing what is known about the pathophysiology of AD and its relevance to the field of molecular imaging.

PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE

Molecular biology has elucidated the biology of Aβ protein turnover: it begins with the production of amyloid precursor protein and its subsequent enzymatic cleavage into Aβ isomers. One of these isomers, Aβ-42, is particularly prone to aggregation and it is thought that its over-production and/or delayed clearance that are responsible for the plaque generation. Variations in the genes governing the various stages of Aβ turnover have been associated with both early- and late-onset forms of the disease, giving credence to the notion that Aβ plays the critical initiating role in the disorder. Specifically, several rare autosomal dominant mutations (PSEN1, PSEN2 and APP genes) have been shown to invariably result in AD onset before the age of 65 through dramatically increased Aβ-42 production. In addition, individuals with an extra APP copy through chromosome 21 trisomy (Down's syndrome) are at a significantly higher risk of developing dementia and invariably have sufficient levels of plaque and NFTs to warrant a pathological diagnosis of Alzheimer's disease by the age of 40 (*5*). Genetic risk of late-onset AD is primarily mediated by the E4 and E2 alleles (increased and decreased risk respectively) of the apolipoprotein gene that governs lipid transfer. In addition to increasing the risk for AD, APOE4 carriership also associates with earlier onset therefore implicating its role in cases with onset before the age of 65 (*6*).

The prominence of Aβ burden in AD led to the development of the so-called amyloid hypothesis: Aβ induces neuronal stress (by mechanisms that remain an area of intense research) with subsequent neuronal degeneration and formation of intracellular paired helical filaments (NFTs) consisting of hyperphosphorylated tau. It follows that hyperphosphorylation of tau is a downstream effect triggered by amyloid-beta cortical burden reaching a critical threshold(*7*). Across the full course of the disease NFTs plaques tend to co-localize more closely with regions of atrophy and hypometabolism than Aβ plaques which has led to the current view that they underlie much of the neurodegeneration found in Alzheimer's disease although there is evidence that they can in turn promote Aβ's own neurotoxic effects (*8*).

Extrapolation of longitudinal Aβ imaging as well as other biomarker data (most notably cerebro-spinal fluid), has led to the conclusion that AD follows a prolonged preclinical course of 15 -20 years of Aβ deposition in the cerebral cortex without any evidence of cognitive impairment (*9*).

As a result Aβ-targeting therapeutic trials in AD have shifted their focus from the syndromic to the preclinical stages of the disease in an attempt to modify Aβ deposition before clinical symptoms are apparent (*10*) in a fashion analogous to the treatment model used to reduce the incidence of coronary artery disease through lipid modification by statins. Such a treatment does not aim to remove the present Aβ plaques but instead attempts to shift the biology towards greater removal or decreased production of the protein. An alternative strategy is to develop compounds interfering with the various stages of NFT formation: tau hyperphosphorylation, microtubule depolymerization and aggregation (*11*). An effective tau directed therapy aims to sever the links between Aβ accumulation and downstream neurotoxicity as mediated by NFTs. A further strategy which is under exploration is to interfere with neuroinflammation which is thought to be a major conduit of the AD protein neurotoxic effects (*12*).

NEUROPATHOLOGICAL ASSESSMENT OF ALZHEIMER'S DISEASE

Until recently the only method to definitively diagnose Alzheimer's disease as a cause of dementia was through post-mortem examination. The standard for this was defined by the National Institute on Aging and Alzheimer's Association (NIAA) (*13,14*). It relies on a tiered evaluation of brain regions for three pathological hallmarks of AD: diffuse Aβ plaque burden (based on Thal phases), NFTs burden (based on Braak stages) and neuritic plaque location and density (based on the CERAD score). Immunohistochemistry is the preferred method for the first two elements while neuritic plaque evaluation technique described in the modified Consortium to Establish a Registry for Alzheimer's Disease (CERAD) protocol recommends thioflavin S or Silver stain (*15*). It should be noted that the distinction between diffuse and neuritic plaques is imperfect as they tend to contain varying levels of Aβ protein which affects their reactivity. Each of the three components ('ABC score: 'A 'for Aβ, 'B 'for Braak and 'C 'for CERAD) is scored 0-3 (based on and translated from the Thal, Braak and CERAD scoring systems respectively) and a final combined score relating to AD pathology is generated, which corresponds to the level of probability (none, low, intermediate and high probability) and which is used to assess the likelihood that AD neuropathologic changes can be considered causative of the cognitive impairment or dementia in a particular patient. The novel scintigraphy methods for assessing AD pathology offer an exciting new opportunity to bring these diagnostic procedures to the living patient with subsequent impact on decision making for disease-modifying therapy should this become available in future.

AMYLOID BETA STAGING

As discussed earlier, the long preclinical stage of AD is characterized by the gradual accumulation of Aβ plaques across the cerebral cortex. This is thought to be a result of imbalance between the production and removal of extracellular Aβ protein. Aβ accumulates in the form of Aβ-only diffuse plaques (amorphous, irregular shaped β Aβ collections lacking surrounding neurites) as well as the combined Aβ-NFT pathology of neuritic plaques.

In 2002, Dietmar Thal and co-authors published autopsy results of 51 carefully selected patients ranging from cognitively normal to severely demented (*16*). The authors identified progressive cerebral changes which were classified into 5 phases of cerebral β-amyloidosis. These stages have subsequently been coined "Thal" phases. The phases are based on a single parameter, the presence or absence of Aβ deposits in specific regions of the brain, without regard to the quantity Aβ present in a location or the type of Aβ plaque (i.e. diffuse or neuritic).

FIGURE 1

In phase 1, sparse Aβ deposits are identified in the frontal, parietal, temporal or occipital cortex (i.e. neocortex), appearing as focal or small groups. No other areas of Aβ plaque deposition are noted. The presence of neocortical Aβ but not hippocampus, entorhinal and midbrain, irrespective of plaque density, is considered phase 1.

There is progressive increase in Aβ deposition in cortical and subcortical regions during phases 2 – 5, beginning with structures of the medial temporal lobe. Phase 2 includes the entorhinal cortex, the CA1 portion of the hippocampus, as well as to a lesser extent the cingulate gyrus, amygdala and extension into the subpial layer in the regions of phase 1.

Progression to phase 3 is characterized by the presence of Aβ plaques in deeper subcortical nuclei including the caudate, putamen, claustrum, thalamus and hypothalamus as well as white matter and greater bandlike deposition in the regions of phases 1 and 2. Phase 4 involves midbrain structures (including the substantia nigra and red nucleus) and the inferior olivary nucleus in the medulla. Finally phase 5 disease includes additional brainstem nuclei as well as the cerebellum.

Subsequent investigations have further elaborated on the Thal staging system with attention to the Aβ plaque density present in each phase. Murray and co-authors evaluated regional distribution of Aβ plaque density in 3618 autopsies in neocortical areas and the hippocampus. In addition to the autopsy results, 35 cases had recent ante-mortem ^{11}C-labeled Pittsburgh

compound B (PiB) PET amyloid Aβ scans available for correlation (*17*). The study demonstrated that between Thal phases 1 and 2 there is pronounced increase in Aβ plaque counts in the association neocortex, reaching a plateau by phase 3 in most areas of the neocortex evaluated (Figure 2). Correlation of the Thal phases with C11-PiB clearly confirms increased Aβ PET signal as individuals progress along the Thal phases. This is demonstrated in Figure 3 which compares representative C11-PiB images over a range of Thal phases. Quantitation of PiB is performed using SUV ratios (SUVR) with cerebellum as reference range. Patients with clinical and pathological diagnoses of Alzheimer's dementia invariably had Aβ deposition patterns consistent with phases 3 -5.

Fig 2.

Fig 3.

Aβ Imaging of Thal Phases

All currently available Aβ PET tracers bind to the fibrillar Aβ structure and thus have specific affinity for both neuritic and diffuse plaques, non-specific binding to cerebral white matter as well as instances of non-AD pathology e.g. amyloid-laden blood vessels as in cerebral amyloid angiopathy (*18,19*). The high affinity to Aβ plaques is highlighted by the strong correlation between 11C-PiB signal and cerebrospinal fluid levels of Aβ-42, thought to underlie much of the plaque formation (*20*). Aβ imaging is capable of distinguishing between lack of Aβ signal (Thal phases 0-1) and presence of neocortical Aβ (Thal phases 3-5) based on visual analysis of the signal generated in the neocortex. Scans for individuals in Thal phase 2 tend to be reported as intermediate in signal strength. Three Aβ tracers have so far been approved by the Food and Drug Administration (*21*): 18F-florbetapir (Amyvid, Eli Lilly/Avid Radiopharmaceuticals), 18F-florbetaben (Neuraceq, Piramal Imaging), 18F-flutemetamol (Vizamyl, GE Healthcare).

A more fine-grained distinction between Thal phases 0-5 using PET β Aβ imaging is not possible due to a number of related factors:

i) Presence of tracer binding non-specific to AD e.g. to amyloid in blood vessels in cerebral amyloid angiopathy or off-target binding to white matter;

ii) Insufficient Aβ density to generate sufficient signal strength;

iii) Areas of distinction between stages 3 - 5 are small subcortical areas and affected by both partial volume effects and interference by white matter non-specific binding.

iv) Aβ PET tracers bind to both diffuse Aβ and neuritic plaques complicating the distinction between Thal and CERAD scores. For example, according to Thal and co-authors, phase 4 is defined by presence of Aβ plaques in the medulla oblongata and red nucleus whereby 'there are often only one to three plaques in the entire anatomic structure' (*16*). In any case, the clinical utility of differentiating accurately between the advanced Thal stages in vivo is questionable.

The evaluation of Aβ pathology brings to light the inherent conflict between pathological findings and scintigraphic signal in general, which applies to all imaging exams. Imaging is limited by a signal threshold below which there is no appreciable visual signal. This threshold is determined not only by the amount of signal present in the substrate and the level of interfering nonspecific activity but also by the spatial resolution and count sensitivity of the imaging system. Aβ binding radiotracers all have a relatively high degree of nonspecific binding to white matter. This leads to difficulties identifying subtle signal changes that may occur between Thal phases. A final barrier to equating scintigraphic and pathological examinations is that unlike PET Aβ signal, Thal staging is not affected by plaque density in the areas of interest.

As a result of the above considerations the outcome of Aβ imaging is currently restricted to labelling scans as 'positive 'or 'negative'. However, as discussed earlier, this exposes the issue of scans reported as' intermediate positivity'. While such results may introduce an unhelpful ambiguity in the clinical settings, it nonetheless reflects the evidence that sub-threshold Aβ burden associates with cognitive change (*22*) while the rate of Aβ accumulation may point to a particularly high-risk group for dementia (*23*).

The clinical application of Aβ PET scanning remains an area of investigation. The current consensus points to value when confirming AD diagnosis (30% of cases undergo a change of diagnosis following Aβ PET) and increasing diagnostic certainty by 60% (*24*). This has led to 'amyloid positivity' (i.e. supra-threshold amyloid burden as evidenced by cerebrospinal fluid or PET) being adopted as a standard inclusion criterion for trials of disease modifying agents in AD (*11*). In addition, a set of clinical scenarios have been highlighted as appropriate for Aβ scintigraphy by a consensus statement (*25*). While PET Aβ is useful in distinguishing AD from non-AD causes of cognitive impairment it is worth noting that among cognitively healthy adults

amyloid positivity ranges from 10% at age 50 to 44% at age 90 (*26*). This difference in prevalence across age group may mean that Aβ PET offers greater diagnostic utility when clarifying the case of cognitive impairment in younger people (*27,28*). In terms of prognostic power, the best evidence is for subjects with MCI having significantly higher progression rates to AD if scintigraphic findings confirm presence of supra-threshold cortical Aβ plaque burden (*29*). More broadly, long-term follow-up data of 'amyloid positive' individuals has shown tendency for greater cognitive decline relative to those with no significant Aβ burden (*30,31*). It has been proposed that in future Aβ burden information will need to be complemented by measures of tau pathology (cerebrospinal fluid or PET) as well as neurodegeneration (MRI, FDG PET) to define the extent to which a neurodegenerative process is due to AD and to provide an indication of its prognosis (*32*).

Neuritic Plaque Density (CERAD staging)

As described earlier, neuritic plaques are Aβ plaques surrounded by dystrophic neurons rich in NFTs. These plaques are strongly associated with postmortem confirmation of AD through the CERAD scoring system (*15*). Specific biomarker targets for neuritic plaque have not been identified but Aβ tracers have been shown to have affinity for both diffuse Aβ and neuritic plaques (*18,33*). This is because the substrate for all known tracers is the beta-pleated sheet conformation of fibrillary β Aβ (*18*). Currently available Aβ PET tracer signal therefore reflects both the diffuse and neuritic plaque burden.

NEUROFIBRILLARY TANGLE STAGING

The landmark investigation of Heiko and Eva Braak on the staging of Alzheimer's disease neurofibrillary tangles (NFTs) was first published in 1991 (*34*).This paper described the progressive and predictable sequence of buildup of NFTs with AD disease progression. There are 6 Braak stages of NFT propagation that are characterized by both the NFTs' location and density. Importantly NFTs evolve separately from amyloid plaques and therefore the topographical distribution of these two AD findings differs considerably.

FIG 4.

The earliest deposition of NFTs is noted in the anterior medial temporal lobe involving the transentorhinal cortex (stage I) and the adjacent entorhinal cortex (stage II). Stage I and II are

termed the 'transentorhinal stages'. These early stages are considered part of the normal cerebral ageing process and findings are present in the non-demented ageing population (*35-37*). For example, one large autopsy study found that NFTs are present in the entorhinal cortex and hippocampus regardless of clinical status at time of death (cognitively healthy, MCI or dementia). In contrast, severe pathologic changes in the inferior-temporal lobe were only present in those with dementia (*36*). Studies in younger individuals (i.e. under the age of 30) have demonstrated the presence of NFTs in the transentorhinal regions from as early as age 6 (*38*). This is evidence that either AD is a lifelong, slowly progressive condition or that the NFT accumulation in these regions is part of non-pathological ageing (*9*).

Stage III and IV are termed the 'limbic stages' and refer the progression of NFT presence to limbic structures (amygdala and hippocampus) and subsequently thalamus. Clinically, the spread of NFTs to stages III and IV is associated with early signs of cognitive impairment.

Stages V (neocortical association cortices) and VI (primary motor, visual and sensory areas) display the end stages of AD with large numbers of NFT throughout the neocortex, corresponding to the neuropathic diagnoses of AD. Cerebral atrophy is present with the greatest destruction in the neocortical association areas (the prefrontal, parietal and temporal lobes), and relative sparing of the primary visual, motor and sensory cortex.

Tau Imaging of Braak Stages

The superior performance of neuropathological Braak staging of AD relative to Aβ neuropathology with regards to clinical diagnosis and level of cognitive impairment is well established (*37,39-41*). In addition, atrophy on MRI co-localizes with NFTs on subsequent autopsy (*40,42*), including instances where clinical subtypes of Alzheimer's disease are concerned (*43*). PET Imaging of tau binding remains investigational, with numerous candidates currently being studied (*44*). Tau ligand design is complicated by the various pathological isoforms of tau and morphology of the fibrillar aggregates present (*45*) whereby compounds sensitive to tau AD pathology show surprisingly variable affinity to depositions in primary tauopathies (*46,47*).

The most widely studied agent T807, now renamed [^{18}F]AV-1451 (Flortaucipir, Tauvid) licensed from Siemens to Eli Lilly was FDA approved in May 2020. Currently 36 clinical trials with 18F-AV-1451 are listed at Clinicaltrials.gov. Recently, post-mortem data confirming tau binding of this agent has been published (*46-49*). Multiple second-generation tau agents under investigation

show substantially less non-specific binding and higher affinities to primary tauopathies and may be useful in identifying earlier Braak stages (*44*). These agents include MK-6240 (Merck & Co), PM-PBB3 (APRINOIA Therapeutics), PI-2620 (Life Sciences) (*50*) and RO-948 (Roche) (*29*).

Initial studies demonstrated that tau PET signal is an accurate predictor of diagnosis and cognitive impairment of AD that is not improved by MRI data and that it predicts rate of atrophy (*51*). Regional tau PET ligand uptake was shown to vary with clinical phenotypes, aggregating to a larger extent in the cortical areas hypothesized to be affected on the basis of the profile of cognitive impairment (*52*). Intriguingly, investigators have demonstrated that tau PET can provide information on the topographical spread of NFTs along the Braak regions thus suggesting a role for the tracer in staging the progress of AD pathology *in vivo* (53). From a clinical perspective, a recent study showed a high degree of concordance between visually rated tau PET scans and the subsequent extent and topography of NFTs (*54*).

SUMMARY:

The post-mortem diagnosis of AD requires the presence of Aβ plaques, tau deposition and neuritic plaques. A new classification system has emerged based on the three components called the "ABC" (Amyloid, Braak, CERAD). Depending on the level of each component, the pathological findings are scored as i) no; ii) low; iii) intermediate, or iv) high likelihood of AD.

Scintigraphic biomarkers can elucidate early (Aβ stages 0-1) and late stages (3-4) presence of Aβ in the form of diffuse as well as neuritic plaques. They therefore reflect burden contributing to both Thal and CERAD staging components. Second generation tau imaging agents with lower non-specific binding may allow discrimination of the Braak stages, likely on a simplified four scale scoring system. These new *in-vivo* biomarkers of AD pathology allow detection of the neuropathic changes of AD ante-mortem. A biological definition of AD based on the presence of Aβ without presence of tau has been proposed to carry the label 'AD pathological change' , and the presence of both sufficient Aβ - and tau signal with or without evidence for neurodegeneration is confirmatory of biological AD (*32*).

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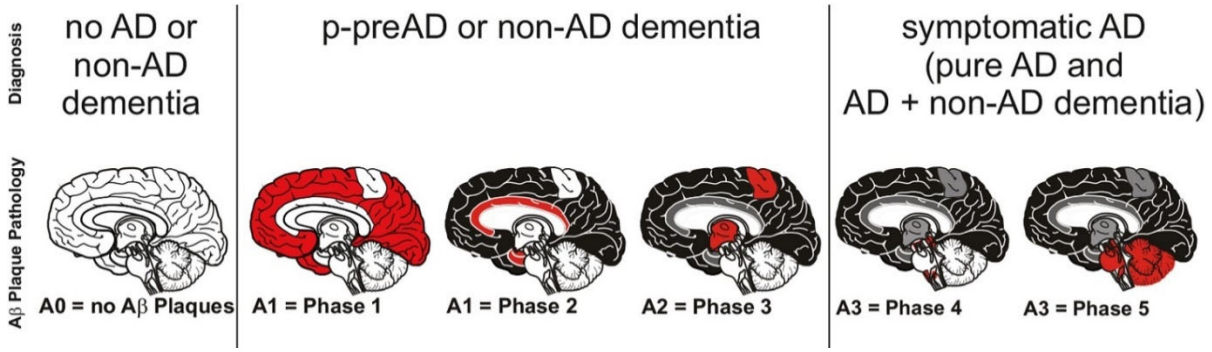

Phase 0	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
No plaque	Sparse, small groups of diffuse neocortical plaque	+ allocortex hippocampus and entorhinal cortex	+ striatum Subpial, post cingulate gyrus	+ Midbrain-substantia nigra, medulla Oblongata	Cerebellum, Reticular Formation of the pons

Fig 1. Representation of Thal phases of Aβ plaque accumulation and their correspondence to clinical status (*55*). Reprint allowed by Creative Commons License.

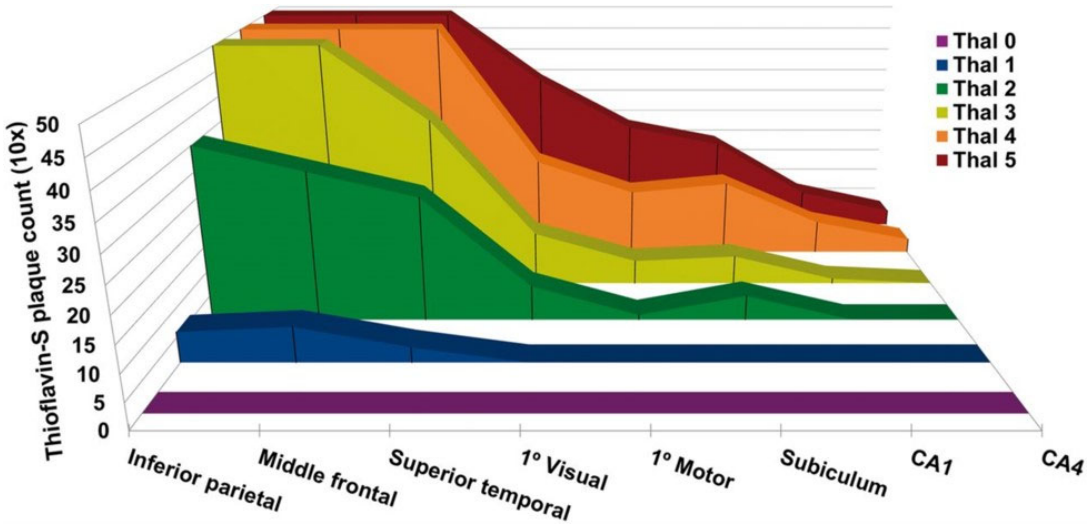

Fig 2. Progression of Amyloid plaque density in Thal stages 1-5 (*17*). Reprint allowed by Creative Common license, Oxford University Press

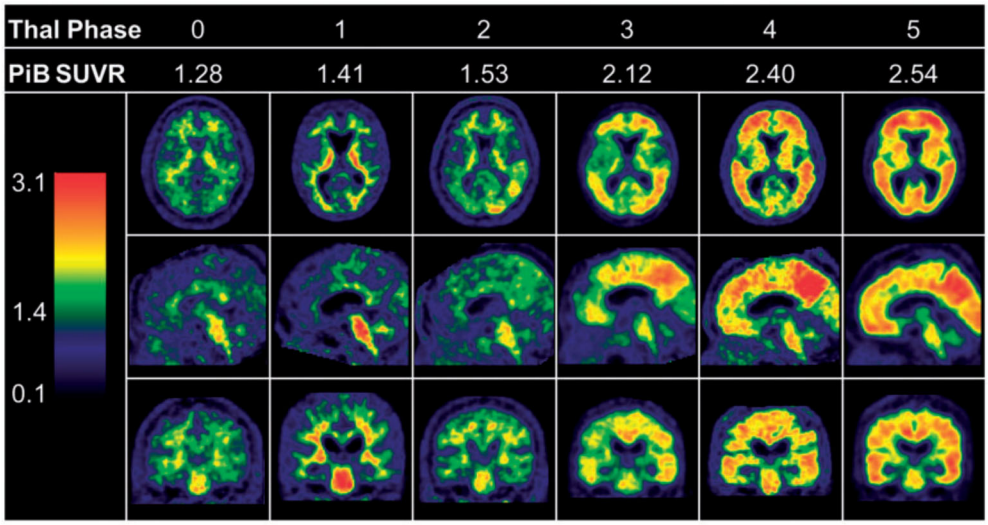

Fig 3. Correlation of Thal stages with amyloid plaque signal as measured by C11-PiB SUVr (*17*). Reprint allowed by Creative Common license

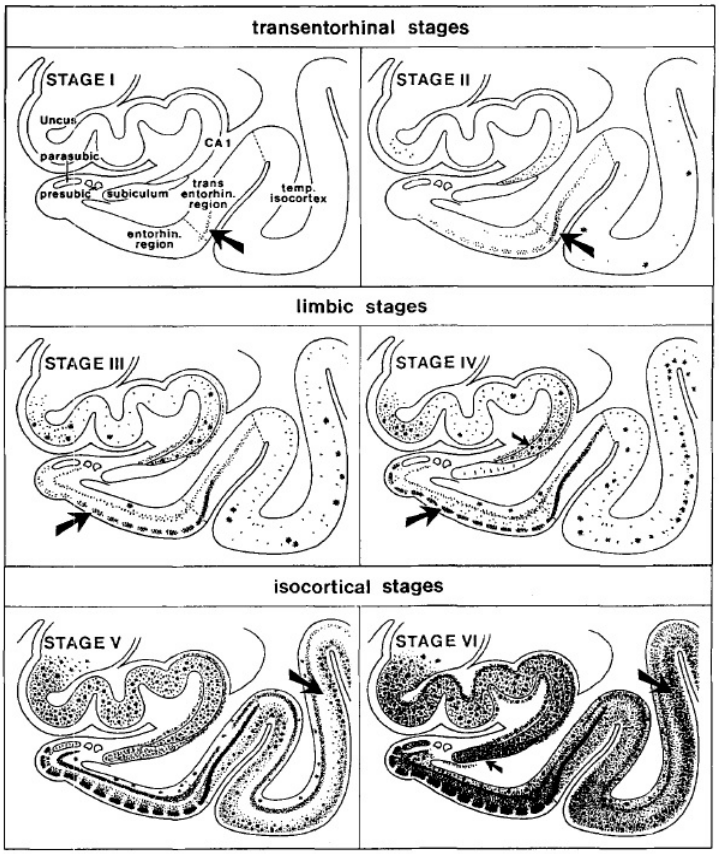

Fig 4: Representation of Braak Stages 1-6 (*56*).