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Clinical Validity of Aß-Protein Deposition Staging in Brain Aging and Alzheimer Disease

GABRIEL GOLD, MD, ENIKÖ KÖVARI, MD, GINA CORTE, MD, FRANÇOIS R. HERRMANN, MD, MPH, ALESSANDRA CANUTO, MD, THIERRY BUSSIÈRE, PHD, PATRICK R. HOF, MD, CONSTANTIN BOURAS, MD, AND PANTELEIMON GIANNAKOPOULOS, MD

Abstract. Braak's neurofibrillary tangle (NFT) pathology staging system of Alzheimer disease (AD) correlates generally with clinical data. Recently, Braak's group proposed an Aß-protein staging based on the progression of amyloid deposition in the medial temporal lobe. To examine its clinical validity and evaluate whether it adds predictive power to NFT-based staging, we performed a study comparing both neuropathological classifications with clinical dementia rating scale (CDR) scores in a large autopsy series. The 2 neuropathological staging systems were strongly correlated. Their association with clinical severity was highly significant. However, the strength of the relationship was greater for NFT-based staging. It accounted for 26.5% of the variability in clinical severity, Aß-protein-based staging for 13.0%, and age for 4.4%. Compared to NFT-based staging, the Aß-protein-based system was less able to distinguish mild cognitive changes from dementia and showed marked overlap among the various stages of cognitive decline. In a multivariate model, NFT and age together accounted for 27.2% of the clinical variability and the addition of Aß-protein deposition staging could only explain an extra 2.9%. Our data support the close relationship between NFT progression and amyloid formation within the medial temporal lobe proposed by Braak's group but demonstrate the limited value of Aß-protein deposition staging in terms of clinicopathological correlations.

Key Words: Alzheimer disease; Amyloid deposits; Clinicopathological correlations; Cognitive impairment; Dementia; Neurofibrillary tangles.

INTRODUCTION

Alzheimer disease (AD) is the most common cause of dementia in the elderly, affecting nearly 10% of the population after 65 yr of age (1, 2). From a neuropathological standpoint, neurofibrillary tangles (NFT) and senile plaques (SP) are the main pathological features associated with AD. These lesions are the basis of currently used neuropathological diagnostic criteria for AD (3). However, they are also present in normal brain aging. Numerous studies of the brains of nondemented elderly people have demonstrated the presence of NFT confined to the temporal neocortex, implying that the progression in NFT density within adjacent components of the medial and inferior aspects of the temporal cortex may take place in cognitively intact individuals (4–10). In addition, SP may appear early in the neocortex of intellectually preserved individuals, whereas the hippocampus is relatively spared by SP formation at the onset of the degenerative process (8, 11–13).

The relationship between the pattern and densities of NFT and SP lesions in brain aging and in AD has led to the development of neuropathological staging models of AD. In 1991, Braak and Braak developed an NFT-based model to differentiate initial, intermediate, and advanced stages of AD (14–18). This model assumes a predictable temporal pattern in the evolution of neurofibrillary changes that can be ordered in a particular regional hierarchy within the medial temporal lobe. In their initial report, these authors suggested that the transentorhinal stages (stages I and II) correspond to a clinically silent initial phase of AD; limbic stages (stages III and IV) to early AD; and neocortical stages (stages V and VI) to fully developed AD (14). Although there is a considerable degree of overlap without a clear-cut threshold between normal and demented cases, the Braak staging scheme appears plausible and correlates well with clinical data in elderly people (19–23). A large body of evidence suggests that NFT but not SP correlate with the severity of dementia (24). However, recent morphological and biochemical studies have challenged this point of view (25, 26). Recently, Braak's group described a new amyloid-based model. Amyloid Aß peptide is the main component of SP. This newer model describes 4 phases in the evolution of amyloid Aß deposition within the medial temporal lobe, which correlate significantly with the Braak NFT based stages (27). In phase 1, diffuse Aß deposits are found in the basal temporal neocortex. In phase 2, diffuse Aß deposits occur within the external entorhinal layers, whereas punctate amyloid deposits (''fleecy amyloid'') are seen in the internal entorhinal layers and CA1 field of the hippocampus. The third phase

From the Departments of Psychiatry (AC, EK, CB, PG) and Geriatrics (FRH, GC, GG), HUG Belle-Idée, University of Geneva School of Medicine, Geneva, Switzerland; Research Center for Neurobiology and Neurobiology of Aging, Fishberg Laboratories (PRH, TB, CB), and Departments of Geriatrics and Adult Development, and Ophthalmology (PRH), Mount Sinai School of Medicine, New York, New York.

Correspondence to: Dr. Gabriel Gold, Department of Geriatrics, University of Geneva Hospitals, 3, Chemin du Pont Bochet, 1226 Thônex—Geneva, Switzerland.

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TABLE 1
Demographic Data in the Present Series

CDR	Mean age in years	(standard deviation)	Number of cases	(women/men)
0	83.3	(9.7)	21	(16/5)
0.5	86.6	(9.1)	30	(18/12)
1	89.3	(7.0)	19	(15/4)
2	91.0	(4.0)	24	(18/6)
3	91.2	(6.2)	59	(45/14)
All cases combined	88.9	(7.7)	153	(112/41)

CDR = clinical dementia rating scale.

is characterized by increased Aß deposition in the molecular layer of the dentate gyrus, entorhinal cortex, and temporal neocortex as well as in the parvopyramidal layer of the presubiculum. In phase 4, diffuse Aß deposits and core-only SP are observed in the CA3–4 hippocampal fields. This sequence of Aß deposition is also followed by neuritic plaques that progressively invade the medial temporal lobe in sites receiving afferent input from NFT-containing neurons (27). The relationship between this amyloid model and clinical staging of cognitive function has not been tested. Furthermore, since this new hierarchical model of amyloid pathology requires an exhaustive and complex morphological analysis of Aß deposits, it is crucial to examine whether it adds predictive power to the NFT-based Braak neuropathological staging. To address these issues, we performed a study comparing both neuropathological classifications (NFT and Aß-protein deposition) with a clinical staging of cognitive functions as measured by the clinical dementia rating scale (CDR) (28) in a large series of autopsied cases with either no cognitive impairment or varying severities of AD.

MATERIALS AND METHODS

The sample included 153 patients aged 63 to 104 yr who died and were autopsied at the Geriatric Hospital of the University of Geneva School of Medicine (Switzerland). The presence and severity of dementia were assessed in all cases with the CDR during the 3 months prior to death. The CDR is a validated scale that is widely used for the clinical staging of dementia (28). It assigns cognitive function to 5 levels defined as no dementia (CDR = 0), questionable dementia (CDR = 0.5), mild dementia (CDR = 1), moderate dementia (CDR = 2), and severe dementia (CDR = 3). Gender and age distribution according to CDR score are listed in Table 1.

The brains were obtained at autopsy (postmortem delay: 3–20 h), fixed in a 15% formalin solution for at least 6 wk, and cut into 1-cm-thick coronal slices. Following macroscopic examination, the left hemisphere of all cases was cut coronally into 1-cm-thick blocks so as to classify them neuropathologically according to Braak and Braak (14) and Thal et al (27). Of these, 3 tissue blocks contained portions of the medial temporal lobe. Subsequently, 2-mm-thick paraffin embedded blocks from the anterior and posterior portions of each block were prepared and cut into 20-μm-thick sections. All sections from the paraffin embedded blocks including the medial temporal lobe, as well as every tenth section in the other paraffin embedded blocks, were processed with fully characterized antibodies to the microtubule associated tau protein (29) and to the core amyloid Aß protein (4G8) (30). Adjacent sections were stained with Gallyas silver stain (31) to identify neuritic plaques. The anti-tau antibody used in the present study was a rabbit polyclonal antibody (961-S28T) raised against a synthetic peptide corresponding to a sequence located in the carboxyl terminal of tau protein (serine 400-threonine 429, according to the numbering of the longest human tau isoform). The 2 putative sites of phosphorylation at serine residues 404 and 422 present in this sequence are found in paired helical filaments. This antibody detects both intracellular and extracellular NFT. The sections were incubated overnight at 4°C with a dilution of 1:4,000 for both antibodies. Following incubation, sections were processed by the PAP method using 3,3′-diaminobenzidine as a chromogen (31). Braak NFT and Aß-protein deposition staging were performed by 2 independent investigators (EK and CB), blinded to the clinical findings, with a high inter-rater reliability (kappa = 0.95). Briefly, for NFT, all cases were classified as belonging to either the transentorhinal (I and II), the limbic (III and IV), or the neocortical stages (V and VI). Thereafter, the exact Braak NFT stage was assessed. The neocortical pathology was estimated in the inferior temporal (Brodmann's area 20), frontal cortex (Brodmann's area 9), and visual association cortex (Brodmann's area 19). Aß-protein deposition staging was performed according to the amyloid nomenclature proposed by Thal et al (27).

Spearman rank correlation coefficients were calculated to measure the association between Aß-protein-based and NFT-based neuropathological staging systems. Maximal likelihood ordered logistic regression with proportional odds was used to evaluate the association between CDR scores and neuropathological classifications in a univariate model. Subsequently, the same method was applied in a multivariate model to take into account the effect of age as well as the interaction between Aß-protein deposition and NFT classifications (32, 33). Since the neuropathological classifications are ordinal scales, they were entered in the models as dummy variables. Maximum likelihood ordered logistic regression can evaluate the relationship between an ordinal outcome variable (CDR) and several independent variables. It was used to calculate the probability of having a specific CDR level as predicted by the neuropathological stage. This method can also evaluate the amount of variability of the outcome variable (i.e. the CDR score) that can be explained by the independent variables (i.e. Aß-protein deposition phase, NFT stage, and age) and thus provide an estimate of the strength of the relationship. In a multivariate model it can estimate the amount of additional information provided by the Aß-protein staging system beyond that already provided by Braak NFT staging. Since there were only 4 cases with a NFT stage of VI, these were combined with stage V cases for the maximum likelihood logistic regression analyses. Statistical analyses were performed using the Stata software package, release 6 (College Station, TX).

RESULTS

The positive association between clinical severity estimated by CDR scores and neuropathological staging was highly significant for both NFT ($p < 0.001$) and Aß-protein deposition classifications ($p < 0.001$). However, the strength of the relationship was greater for NFT-based Braak staging (Fig. 1). In a univariate model, it accounted for 26.5% of the variability in clinical severity as measured by the CDR, whereas Aß-protein deposition staging accounted for 13.0% and age for 4.4%. Examination of these data reveals areas of overlap between clinical and neuropathological severity (Fig. 2). For the NFT classification, patterns were similar in cognitively intact cases (CDR 0) and cases with very mild cognitive changes (CDR 0.5). Stage III was associated with all CDR levels; however, stage IV was consistently associated with at least mild dementia (CDR 1 or higher). Furthermore, moderate and severe dementia (CDR 2 and 3) corresponded almost exclusively to Braak stage III or greater. Likewise, the Aß-protein-based classification exhibited similar patterns for CDR 0 and CDR 0.5, but it was also less able to distinguish mild cognitive changes from dementia, and showed marked overlap among the various stages of cognitive decline. Clinical severity probabilities were computed for each neuropathological stage using both the NFT and Aß-protein deposition classifications (Table 2). Normal cognition or mild cognitive impairment (CDR 0 and 0.5) were more likely with early NFT stages than early Aß-protein deposition stages. Severe cognitive deficits (CDR 2 and 3) were more likely at advanced NFT stages than advanced Aß-protein deposition stages.

There was a substantial correlation between NFT and Aß-protein deposition classifications (Spearman $r = 0.58$; $p < 0.001$). A multivariate model that included the 2 neuropathological classifications and age explained 30.1% of the variability in CDR scores. However, NFT and age together accounted for 27.2% and the addition of Aß-protein staging to the model could only explain an extra 2.9% of the clinical variability.

DISCUSSION

The present data derive from a large series of autopsy cases with well-documented cognitive status. Other strengths include the rigorous statistical methodology and the fact that neuropathological staging was performed by raters who were blinded to the cognitive status. Although hospital-based cohorts are never fully representative of the entire aging population, it is important to note that our cases spanned a broad age range of geriatric patients and that all levels of cognitive function and all neuropathological staging levels with the exception of NFT stage VI were well represented. Since our series includes older individuals recruited in a geriatric setting, it may not apply to early onset AD.

NFT and Aß-protein Deposition Staging-Based Clinicopathological Correlations in Brain Aging

The positive association between amyloid deposition staging and the clinical severity of cognitive symptoms reported here suggests that the sequential progression of Aß-protein deposition from the inferior temporal neocortex to all hippocampal subdivisions proposed by Braak's group is generally related to the clinical symptoms of Alzheimer disease. However, clinical expression does not correlate closely with the progression of amyloid pathology within the medial temporal lobe. This is particularly striking for phases 1 and 2, which are essentially indistinguishable in terms of CDR score. Inversely, CDR 0 and 0.5 cases displayed the same patterns of Aß-protein deposition with the vast majority of these cases being in phases 1 and 2. Consistent with our previous study (23), Braak NFT stages I and II were also clinically similar, and CDR 0 and 0.5 cases exhibited comparable patterns of NFT involvement in the present series. Altogether, these observations indicate that the first steps of the neurodegenerative process described by Braak's group both for NFT and Aß deposits do not correspond to a stepwise decrement of cognitive functions. Moreover, the occurrence of questionable dementia does not reflect a consistent worsening of NFT and Aß deposits pathology within the medial temporal lobe (34). This could be partly explained by the marked clinical heterogeneity of CDR 0.5 cases. Morris et al proposed a division of CDR 0.5 group into CDR 0.5/AD, CDR 0.5/incipient AD, and CDR 0.5/uncertain AD subgroups, and demonstrated that the progression to greater dementia severity in CDR 0.5 patients

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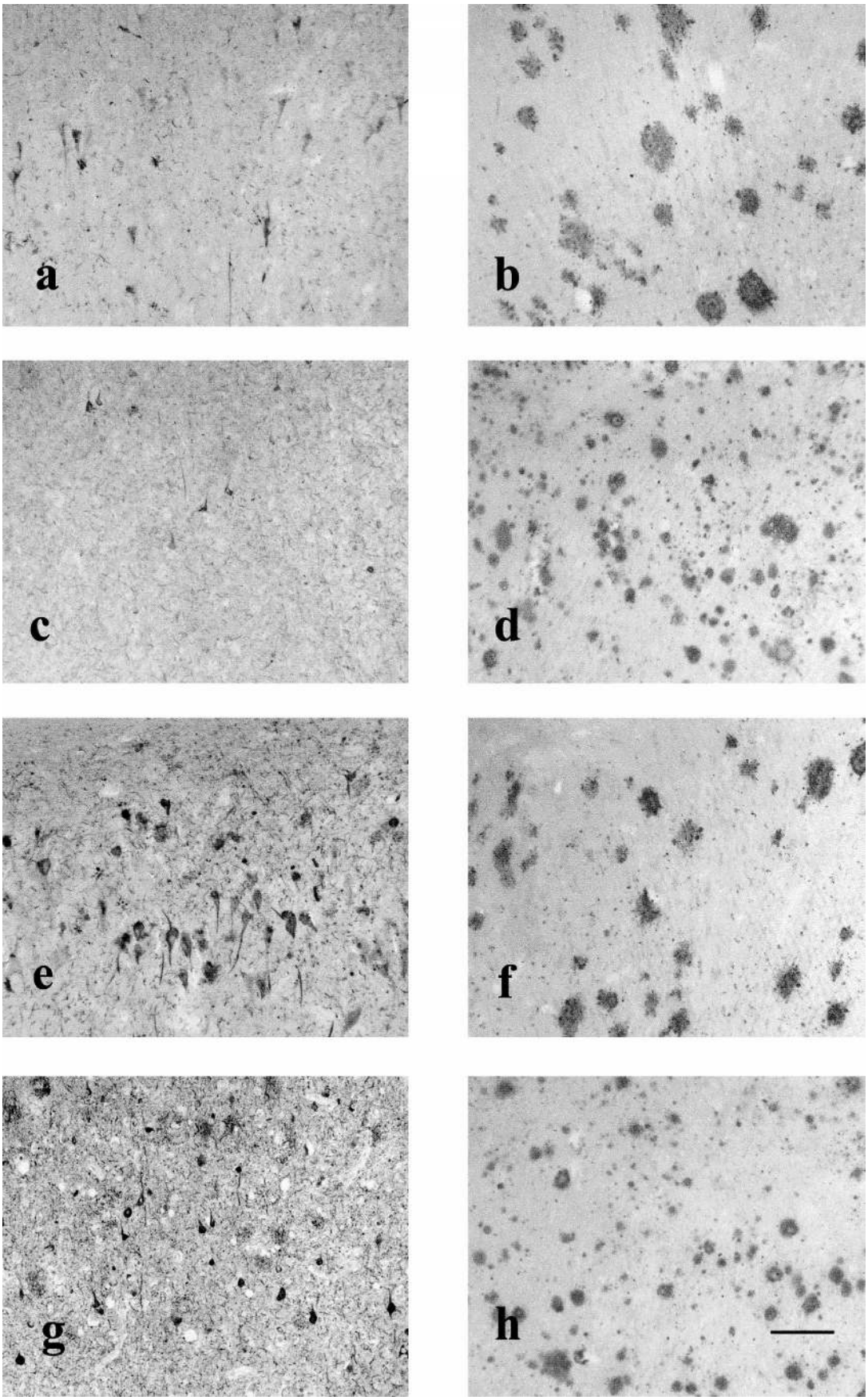
Fig. 1. Examples of NFT (a, c, e, g) and senile plaque (b, d, f, h) distribution in the CA1 field of the hippocampus (a, b, e, f) and Brodmann's area 20 of the mid temporal neocortex (c, d, g, h) in a CDR 0.5 case (a–d) and a CDR 3 case (e–h). The CDR 0.5 case had a Braak stage III for NFT and the CDR 3 case a Braak stage V. Note the considerable increase in NFT density in the CDR 3 case in both the CA1 field (e) and the temporal neocortex (g). In contrast, apparent differences in senile plaque densities are more difficult to assess between the CDR 0.5 case (b, d) and the CDR 3 case (f, h) in either region. The plaques appear larger overall in the CDR 0.5 case compared to the CDR 3 case, and the area occupied by amyloid deposition in these microscopic fields does not differ considerably between these 2 cases. Based on the Braak staging system for amyloid deposition, both cases were rated as phase 3. Note the overall strong Aß protein immunoreactivity in all of the deposits, the occurrence of many classical plaques, and the absence of lightly stained ''fleecy'' amyloid in both regions. In each case and each region, sections stained for amyloid and tau protein were obtained from the same blocks of tissue and were immunoreacted in the same experiment. Scale bar (shown in part [h]): a, c, e, g = 100 μm; b, d, f, h = 200 μm.



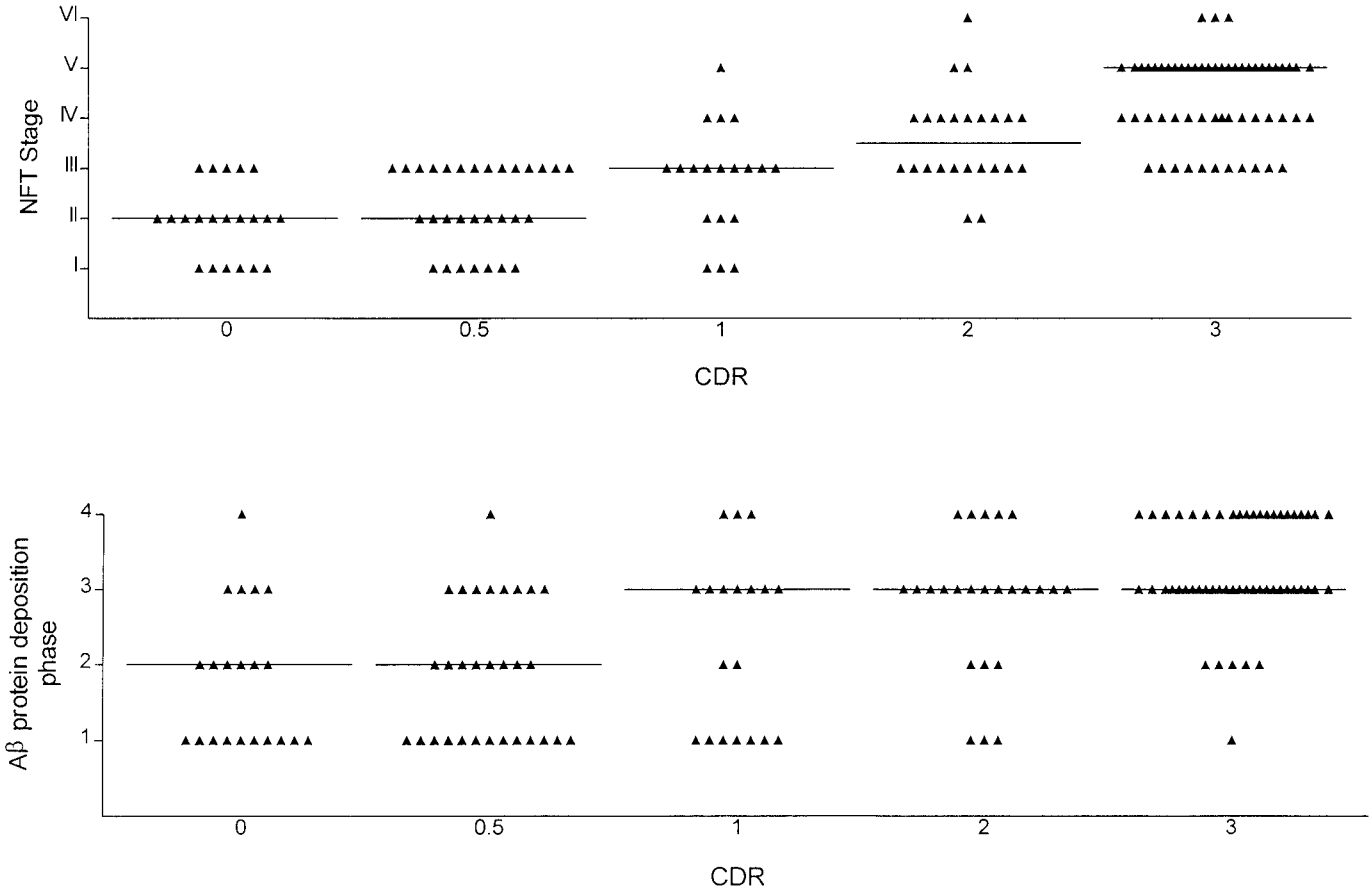


Fig. 2. Column scatter plots of NFT stage (top graph) and Aß-protein deposition phase (bottom graph) according to CDR scores. Each triangular symbol corresponds to an individual case and horizontal lines represent the median neuropathological stage for each CDR level.

TABLE 2
Clinical Severity Probabilities as a Function of Neuropathological Staging

	NFT Braak					Aß-protein deposition staging			
CDR	I	II	III	IV	V–VI	1	2	3	4
0	0.41	0.41	0.08	0.01	0.00	0.33	0.22	0.06	0.02
0.5	0.40	0.39	0.26	0.05	0.01	0.34	0.32	0.14	0.05
1	0.12	0.12	0.25	0.09	0.02	0.14	0.17	0.14	0.06
2	0.06	0.06	0.25	0.23	0.08	0.10	0.14	0.22	0.14
3	0.03	0.03	0.17	0.63	0.88	0.09	0.14	0.45	0.73

Results represent the probabilities of each CDR score for a given neuropathological stage. See text for details.

depends on the degree of cognitive impairment at baseline (35). Alternatively, the poor performance of the neuropathological staging systems in patients with a CDR score of 0.5 may be due to an underestimation of the complexity of pathologic changes occurring in the early stages of the dementing process. Previous studies have shown that at least some of the CDR 0.5 cases display a substantial number of neurons in transition to NFT as well as a significant neuronal depletion in the entorhinal cortex (36–38). Recently, a longitudinal clinicopathological study demonstrated that a substantial proportion of older adults meet the Khachaturian and CERAD neuropathological criteria for AD without significant changes in mental status measures (39). On the basis of these observations, the authors proposed the presence of a brain reserve allowing for normal cognitive functioning in individuals with significant neuropathological changes within the medial temporal lobe. This possibility is further supported by a biochemical and morphological study that revealed elevated synaptic protein levels in the neocortex of very mild AD cases, and proposed that a transient adaptive synaptic response may occur in the earliest clinically detectable stages of dementia (40). It is therefore possible that both NFT and Aß-protein deposition staging systems are insufficient to establish valid clinicopathological correlations in the initial phases of the degenerative process since they do not take into account neuronal death, early-stage NFT development in the entorhinal cortex, compensatory brain responses, or threshold effect.

The substantial development of neuritic plaques in phases 3 and 4 is associated with a shift towards higher CDR scores. However, 20% of phase 3 cases and 7% of phase 4 cases displayed CDR scores of 0 or 0.5, pointing to the difficulty in establishing a precise neuropathological cut-off point for clinical dementia based on Aß-protein deposition staging. Furthermore, all Aß-protein deposition phases are represented among cases with CDR 2 and 3. In particular, 19% of phase 1 cases and 28% of phase 2 cases presented with moderate to severe dementia, suggesting that this staging system does not reliably predict the severity of cognitive decline in overtly demented patients. This agrees with several previous quantitative analyses which showed that amyloid deposition is a poor correlate of clinical status in moderate and severe AD cases (6, 11, 41). It is possible that resorption of beta-amyloid may surpass its deposition in end-stage AD as suggested in a previous study by Thal et al (42), which demonstrated that SP stages and the degree of cortical microglia reaction increased up to Braak NFT stages IV and V but tended to decrease in more severe cases.

Clinical Validity of Aß-Protein Deposition Staging

A crucial issue for the implementation of a new neuropathological staging system is its clinical validity. In this respect, our study indicates that the newly proposed amyloid deposition staging does not achieve the performance of the Braak NFT staging. Despite generally similar distribution patterns across the different CDR scores, NFT stages were more closely associated with CDR scores than were Aß-protein deposition phases. In a univariate model, the strength of the relationship with CDR scores was much higher for NFT staging compared to the Aß-protein-based classification. More importantly, the multivariate analysis demonstrated that Aß-protein deposition staging provided minimal additional predictive power beyond that already provided by NFT staging. These results parallel the observations of Grober et al (22) who showed, in a prospective series, that adjustment for Braak NFT stages eliminates the association between cognitive measures and amyloid burden. From a neuroanatomical viewpoint, these findings as well as the strong correlation between NFT and Aß-protein deposition staging reported in the present series confirm the observations of Thal et al (27) giving additional support to a chronological link between the 2 types of lesions (5, 16, 43, 44). From a clinicopathological viewpoint, they are consistent with several earlier reports (5, 8, 9, 11, 45–47) suggesting that the age-associated sequential spreading of Aß deposits from basal temporal areas to the entorhinal hippocampal pathways has limited clinical relevance.

Conclusions

In our experience, the Aß-protein deposition staging performed significantly less well than NFT staging in explaining the variability of the cognitive status, at least for older hospitalized individuals. Furthermore, the use of both staging systems only marginally improved the predictive value of NFT staging. Thus, the added complexity of the Aß-protein deposition staging system is not counterbalanced by a significant gain in terms of clinical validity compared to the traditionally used Braak NFT classification. Our data support the close neuroanatomical relationship between NFT progression and amyloid formation within the medial temporal lobe proposed by Braak's group, but question the usefulness of Aß-protein deposition staging, at least for clinicopathological correlations. Further neuropathological studies in community-based samples, including stereological estimates of neuronal loss in the hippocampal formation and entorhinal cortex, immunocytochemical assessment of both transitional and end-stage NFT, as well as measures of synaptic protein levels, will be instrumental in improving the functional validity of lesion-based staging systems in brain aging and dementia.

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