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Clinical validity of Braak neuropathological staging in the oldest-old

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Abstract Several studies have demonstrated a good correlation between clinical severity and Braak's neuropathological staging in Alzheimer's disease (AD). However, nonagenarians and centenarians display a different pattern of cortical vulnerability to the neurodegenerative process compared to younger elderly, and it is not known whether correlations between clinical severity and neuropathological stages remain valid in this age group. To address this issue we compared Clinical Dementia Rating scale (CDR) scores and Braak stages in 116 patients over 90 years of age with either no cognitive impairment or very mild to severe AD. There is a strong positive correlation between CDR scores and Braak staging (Spearman coefficient = 0.66; $P < 0.01$). However, neuropathological staging does not distinguish cases with normal cognition (CDR 0) from those with mild cognitive changes (CDR 0.5). Unlike younger cohorts, Braak stages I and II are frequently associated with questionable dementia in this age group. Braak stage III overlaps with all CDR levels and correlates poorly with cognitive function. Braak stages IV or greater are consistently associated with at least mild dementia. Consistent with our previous neuropathological analyses of nonagenarians and centenarians, the present data suggest that the substantial involvement of the hippocampus which characterizes Braak stage IV is a key step in the development of overt clinical signs of dementia in the oldest-old. Moreover, they indicate that Braak staging represents a broad concept of the evolution of neurofibrillary tangles rather than a precise hierarchical model associated with a stepwise deterioration of cognitive abilities near the upper limit of life.

Key words Alzheimer's disease · Clinicopathological correlations · Cognitive impairment · dementia · Neurofibrillary tangles

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Introduction

Alzheimer's disease (AD) is characterized clinically by progressive deterioration of higher cortical functions and neuropathologically by neurofibrillary tangles (NFT), senile plaques, and neuronal and synaptic loss [11]. Braak and Braak [3, 4] developed a neuropathological staging model to differentiate initial, intermediate and advanced stages of AD. This model presupposes a predictable temporal pattern in the evolution of neurofibrillary changes that can be ordered in a particular regional hierarchy. In stages I and II, the transentorhinal region is preferably affected with only mild involvement of the hippocampus, the neocortex is essentially spared. Stages III and IV, referred to as limbic stages, include more abundant NFT in the transentorhinal and entorhinal cortex, moderate involvement of the hippocampus and only mild neocortical pathology. Cases with stages V and VI display high NFT densities in both the hippocampus and neocortex. In their initial report, Braak and Braak [3] suggested that the transentorhinal stages (I and II) correspond to a clinically silent initial phase of AD, limbic stages to early AD, and neocortical stages (V and VI) to fully developed AD. However, these authors did not correlate neuropathological findings with the severity of cognitive decline. Several recent studies have attempted to address this issue [1, 2, 5, 7, 8]. Although there is a considerable degree of overlap without a clear-cut threshold between normal and demented cases, the Braak scheme appears plausible and

correlates well with clinical data in elderly people aged below 90 years [14]. However, there is no study, to our knowledge, which demonstrates the clinical validity of Braak staging beyond this age. Several lines of evidence suggest that the regional vulnerability to NFT changes substantially after 90 years of age, in that AD symptomatology depends on the degree of compromise of the anterior CA1 field of the hippocampus [10]. To examine whether the precise neuropathological hierarchy proposed by Braak and Braak corresponds to stepwise decrements in cognitive function in the oldest-old, we performed a prospective study comparing neuropathological Braak stages with a clinical assessment of cognitive functions as measured by the clinical dementia rating scale (CDR) [13] in 116 autopsied nonagenarians and centenarians with either no cognitive impairment or very mild to severe AD.

Materials and methods

The sample included 116 patients aged 90–104 years, who were autopsied in the Geriatric Hospital of the University of Geneva School of Medicine (Switzerland). Cases with major neuropsychiatric illness, alcoholism, cerebral vascular lesions or non-Alzheimer dementias were excluded. Demographic data are presented in Table 1. The presence and severity of dementia was assessed in all cases with the CDR, at least once during the 3 months prior to death [13].

The brains were obtained at autopsy (post-mortem delay: 3–20 h), fixed in a 10% formalin solution for at least 6 weeks, and cut into 1-cm-thick coronal slices. Following macroscopic examination, three blocks were taken to classify all cases neuropathologically according to Braak and Braak [3]. The first block was prepared at the level of the uncus to display the entorhinal and transentorhinal region, the second at the level of the lateral geniculate body to identify the mid level of the hippocampus and inferior temporal cortex and the third at the level of the occipital pole [3]. Subsequently, paraffin-embedded blocks were cut into 20-μm-thick sections which were processed with highly specific and fully characterized antibodies against the microtubule-associated tau protein, and the core-amyloid Aβ protein as previously described [20]. The anti-tau antibody used in the present study was a polyclonal antibody (961-S28T) raised against a synthetic peptide corresponding to a sequence located in the C terminus of tau protein (Ser 400–Thr 429). This sequence contains two putative sites of phosphorylation Ser-Pro at Ser residues 404 and 422 that are found in paired helical filaments [6]. This antibody detects both intracellular and extracellular NFT. The monoclonal anti-Aβ antibody 4G8 is directed against amino acids 17–23 of the β-protein domain. Previous studies have shown that this antibody is very sensitive for demonstrating Aβ deposition in aging brains, and gives consistent and reproducible standardization results for quantification purposes [16, 18]. Braak staging was performed by two independent investigators (E.K. and C.B.), blind to the clinical findings, with a high inter-rater reliability (kappa = 0.95). The following severity scale was applied by both neuropathologists for NFT densities: minimal (< 2 lesions/mm^2), moderate (2–10 lesions/mm^2), abundant (> 10 lesions/mm^2). 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), and, thereafter, the exact Braak stage was assessed. Importantly, the inter-rater reliability was also high (kappa = 0.95) when only stages III and IV were considered. The neocortical pathology was estimated in the inferior temporal (Brodmann's area 20), and visual association cortex (Brodmann's area 19).

Spearman correlation coefficients were calculated between clinical CDR scores and Braak neuropathological stages.

Table 1 Age, gender and CDR scores (*CDR* clinical dementia rating)

CDR score	Mean age (standard deviation)	Number of cases (women/men)
0	91.2 (2.8)	11 (9/2)
0.5	91.7 (2.6)	16 (12/4)
1	92.8 (2.5)	14 (13/1)
2	92.2 (2.2)	21 (18/3)
3	92.9 (2.7)	54 (42/12)
All cases combined	92.5 (2.7)	116 (94/22)

Results

Braak stages and CDR scores were highly correlated (Spearman coefficient = 0.66; $P < 0.01$) with initial neuropathological stages corresponding to early clinical stages. However, examination of the data reveals several areas of overlap between the two staging models (Fig. 1). Among CDR 0 cases, 18% were Braak stage I, 27% were stage II and 55% stage III; for CDR 0.5 cases the distribution was 13% stage I, 31% stage II and 56% stage III.

Thus, neuropathological staging was unable to distinguish cases with normal cognition (CDR = 0) from those with very mild cognitive changes (CDR = 0.5). None of these cases were classified as stage IV or greater.

CDR 1 cases ranged from stage I to stage V with close to half the cases (43%) corresponding to stage III disease. CDR 2 cases ranged from stages II to V with 43% of the cases corresponding to stage III and the same number corresponding to stage IV. However, 95% of CDR 2 cases presented with stage III or higher. Early Braak stages I and II were usually not clinically silent since 71% of stage I and 75% of stage II cases displayed a CDR of 0.5 or

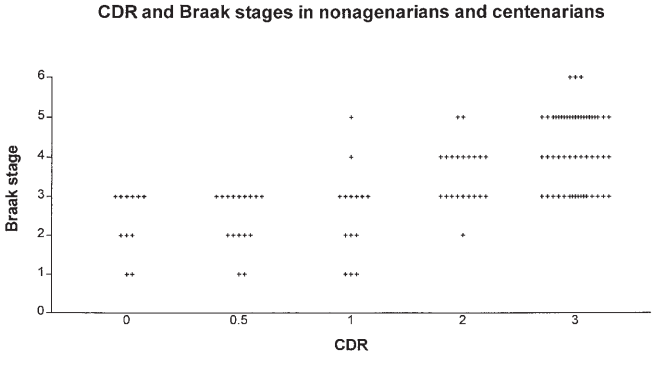

Fig. 1 Column scatter plot of Braak neuropathological stages according to CDR scores. Note the good overall correlation between the two staging models. Note the similar patterns of Braak staging in cognitively intact cases (CDR of 0) and cases with very mild cognitive changes (CDR score of 0.5). Note also the substantial overlap of Braak stage III with all CDR levels. Braak stage IV is consistently associated with at least mild dementia. Moderate and severe dementia (CDR 2 and 3) correspond almost exclusively to Braak stage III or greater. See text for details (*CDR* clinical dementia rating)

greater. However, these stages were incompatible with very severe AD cases (CDR 3), which corresponded mostly to stages III (30%), IV (26%) and V (39%).

Braak stage III overlaps with all CDR levels and correlates poorly with cognitive function (Fig. 1). However, once Braak stage IV is reached, the severity of NFT formation becomes incompatible with a CDR of 0.5 or less. In other words, the transition between Braak stages III and IV implies a clinical transition from normal or questionable cognitive dysfunction to dementia. However, the converse is not true since many cases with a CDR of 2 and several cases with a CDR of 3 corresponded to a neuropathological stage of III or less. The transition from Braak stage IV to stage V implies another increment in clinical severity, since 88% of the cases in the latter neuropathological stage had severe dementia (CDR 3).

To further understand why Braak stage IV was clearly associated with overt clinical dementia, while this was not the case for Braak stage III, we performed a quantitative assessment of NFT densities in the CA1 field, subiculum, entorhinal cortex, and Brodman's area 20 in cases with Braak stages III and IV. In Braak stage III, mean NFT densities per mm^2 were 13.0 ± 2.5 in the CA1 field, 10.6 ± 1.9 in the subiculum, 22.0 ± 2.0 in the entorhinal cortex, and 4.7 ± 1.6 in Brodman's area 20. These values were 27.7 ± 2.5, 14.3 ± 1.4, 24.5 ± 3.2, and 7.1 ± 2.3, respectively, in the Braak stage IV group. NFT densities in the CA1 field were significantly higher in the Braak stage IV compared to the Braak stage III group ($P < 0.01$). In both Braak stage III and IV cases, there was a substantial variability in NFT counts, mainly in the CA1 field and entorhinal cortex.

Discussion

Although the present data reveal a significant correlation between neuropathological Braak stages and CDR scores in very old individuals, as reported in younger elderly cohorts [1, 2, 5, 15], close examination demonstrates that this correlation is not linear. Braak stage II can be distinguished from stage I by an increased density of NFT lesions in the transentorhinal area and very mild involvement of the hippocampus, yet this neuropathological progression did not reflect clinical progression of disease in this age group. Our data support regrouping these first two transentorhinal stages as suggested by Braak and Braak [3]. However, stages I and II are not necessarily clinically silent in centenarians since most cases displayed either questionable or mild dementia. The absence of clinical distinction between Braak stages I and II may be due to low sensitivity of the CDR in detecting early cognitive changes in the oldest-old, but this is unlikely since the CDR has been used effectively in a cohort of centenarians [19].

Recent studies have shown that at least some of the cases with a CDR of 0.5 display a substantial number of neurons in transition to NFT [12, 17]. These neurons are enriched in neurofilament protein, a cytoskeletal protein which has been implicated in NFT formation [17, 21, 22]. In addition, estimates of neuronal loss in AD have demonstrated a significant neuronal depletion in the entorhinal cortex of centenarians [10]. Therefore, Braak staging may underestimate AD pathology in the initial phases of the degenerative process, corresponding to CDR 0.5 to 1, by not taking into account neuronal death and early-stage NFT development in the entorhinal cortex.

Several prior studies in younger elderly cohorts failed to identify a clear relationship between stages III and IV and cognitive abilities and have suggested that neither stage is a reliable indicator of clinical status [1, 2, 15]. In our experience, stage III is associated with a full range of cognitive function from normal to severe impairment and, thus, represents a transitional stage. In contrast, stage IV, which is characterized by more severe involvement of the hippocampal formation and mild involvement of the neocortex, represents a neuropathological threshold beyond which dementia is always present in very old people.

It is unlikely that the variability in NFT densities observed in the CA1 field and entorhinal cortex in stage III is sufficient to explain the marked differences in clinical expression since, in fact, such variability was also present in our Braak stage IV cases, although their clinical status was much more homogeneous. Importantly, the quantitative analysis showed that a significant increase in NFT densities in the CA1 field characterizes Braak stage IV, compared to Braak stage III, in very old individuals. This is consistent with previous reports on brains of nonagenarians and centenarians showing that NFT densities in the CA1 field are a strong correlate of dementia [9, 10]. In these studies, very old individuals display no signs of cognitive impairment despite significant NFT formation in several hippocampal subdivisions other than the CA1 field, indicating that a hierarchical model of cytoskeletal pathology is not sufficient to explain the cognitive decline in this age group [9, 10]. Moreover, our results agree with the findings of Gertz et al. [7] in a very elderly community sample of 42 cases aged 81–98 years. In their series, 10% of stage I and II cases were demented, 74% of stage IV or greater cases were demented and stage III cases were intermediate (43% demented). In the Braak classification, stages III and IV are grouped as limbic stages. Our data indicate that the clinical findings associated with stage IV neuropathological changes are markedly different from the clinical expression of stage III and, thus, do not support merging theses two stages in this age group.

Based on our observations, a simplified version of Braak criteria for nonagenarians and centenarians could be proposed including three critical steps. The first step (Braak stages I and II) corresponds to elderly individuals with no or mild cognitive changes. The second transition step (Braak stage III), represents a gray area without precise clinical correlates. The third step (Braak stage IV or greater) is clearly related to the clinical expression of AD. Further studies including both stereological estimates of neuronal loss in the hippocampal formation and entorhinal cortex as well as immunocytochemical assessment of both transitional- and end-stage NFT will be instrumental

in differentiating Braak stages I and II, and clinically defining homogeneous subgroups within Braak stage III in nonagenarians and centenarians.

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