Eur Arch Psychiatry Clin Neurosci (1996) 246:132–136

SPECIAL ISSUE

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The relationship between clinical dementia and neuropathological staging (Braak) in a very elderly community sample

Abstract The neuropathological staging model proposed by Braak and Braak (1991) implies that the evolution of neurofibrillary pathology follows a predictable sequence and can be ordered in a regular regional hierarchy. A total of 42 cases of an elderly population sample, which had been prospectively clinically assessed, were examined. Clinical diagnosis was made according to the CAMDEX criteria, and the sample reported here did not include cases were vascular dementia according to the criteria proposed by Chui et al. (1991). The neuropathological staging procedure was applied as originally proposed by Braak and Braak (1991). In addition, in all cortical laminae and regions which are essential for the staging model neurofibrillary tangles were quantified. Demented cases had significantly more areas involved and more advanced neuropathological stages. Cases with stages 1–3 tended to be non-demented, and cases with stages 4–6 tended to be demented. However, there was a considerable degree of overlap and no clear-cut threshold could be established. This brings into question the diagnostic value of the staging model.

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Key words Aging · Neuropathological staging · Alzheimer's disease · Neurofibrillary tangles · CAMDEX

Introduction

In his original descriptions of the disease which was later named after him, Alzheimer (1907) regarded the neurofibrillary tangle (NFT) as a specific morphological structure that was associated with a dementing process. He did not mention the distribution of this newly discovered lesion. Simchowicz (1911) was the first to study plaques and tangles systematically and in part quantitatively in a larger series of demented cases and controls. He came to the conclusion that the allocortex was most severely affected with NFT, the association cortex has an intermediate position, and that the primary visual cortex had only been affected in very severe cases. Esiri et al. (1986) have shown that also the primary auditory cortex shows NFT only in cases with very advanced pathology. Gellerstedt (1933) as well as Hirano and Zimmerman (1962) came to a further refinement of the distribution of NFT in the allocortical areas. They noticed that even in clinically normal old people, many had NFT in the entorhinal cortex and/or in the hippocampus. Braak and Braak (1991) reexamined and summarized the regional pattern of NFT distribution and came to the formulation of a neuropathological staging model of this pathology. Initially, the staging concept was not linked to any implications about the development of clinical symptoms.

The staging model suggests a regular regional hierarchy in the evolution of neurofibrillary pathology with brain regions lower in the hierarchy, having both fewer tangles, and being affected only in more severe cases than regions that are at a higher position in the hierarchy. The following hierarchical anatomical sequence in the cortex was suggested: the transentorhinal lamina pre-alpha of the transenthorhinal cortex, the lamina pre-alpha of the entorhinal cortex, the hippocampal area CA1, the lamina pri-alpha of the entorhinal cortex, the association cortex

together with lamina pre-β of the entorhinal cortex, the parastriate, and the striate cortex (Brodman areas 18 and 17). According to the hierarchical model, regions that appear earlier in this list have both more tangles and are affected in less severe cases than regions that appear later in the list.

Six stages of evolution were proposed. The first two stages are characterized by the presence of neurofibrillary tangles in the pre-alpha layer of the transentorhinal cortex, occasional tangles in CA1, and none in neocortical regions. Stages 3 and 4 (limbic stages) are characterized by more severe neurofibrillary pathology in transentorhinal and entorhinal cortices and in CA1 of hippocampus, together with the appearance of tangles in the association neocortex. In the isocortical stages (stages 5 and 6) there were numerous tangles in all neocortical association areas and also in the primary sensory areas such as the striate cortex. No distinction was proposed between different lobes and regions of the association cortex.

The neuropathological staging system is based on semi-quantitative judgments of the severity of NFT pathology in a set of defined brain regions. However, it has been possible to prove the validity of this hierarchical model by operationalizing it in quantitative terms (Gertz et al. in press). For that purpose, tangles were counted in all brain regions used for staging. There was a general tendency for NFT counts to decrease according to the sequence predicted by Braak and Braak (1991). The proposed neuroanatomical hierarchy was broadly supported in that 81% of the cases had only one order violation or less. However, there was a broad range of variability especially in area CA1 of hippocampus with values exceeding the corresponding values observed in the entorhinal cortex. The allocortical regions therefore appear to be more variable than suggested by a simple hierarchical model of disease progression. Another important finding of our previous study was a statistically significant positive correlation between the sum of tangle counts from all brain regions and the neuropathological stage. Similarly, there was a significant positive correlation between the number of areas affected and the neuropathological stage. This implies that disease progression, as defined by the neuropathological staging criteria, is strongly associated both with involvement of more brain regions and a higher overall tangle counts in these brain regions.

The purpose of the present study was to analyze the relation between the neuropathological stages and the clinical diagnosis of dementia. The well-established link between tangle pathology and clinical dementia (Ball 1976; Wilcock and Esiri 1982) makes it tempting to look for a neuropathological threshold definable in terms of the staging system, which corresponds to the threshold for the appearance of clinical dementia. If this were indeed possible, it would be a great help in finding a solution to the extremely difficult problem of defining the neuropathological criteria for a diagnosis of Alzheimer's disease, which has not been resolved so far.

The present analysis is based on an epidemiological study of cognitive function in an elderly population in the Cambridge area in which brain tissue was collected irrespective of severity of cognitive impairment, which had been determined prospectively (Paykel et al. 1994). The design of the clinical studies thus made it possible to obtain cases at early and intermediate stages of both cognitive decline and with NFT pathology suitable for testing the hierarchical model.

Materials and methods

The 50 brains analyzed initially are a subset of brains obtained from a population-based sample of people aged 75 years and over in the city of Cambridge, UK (O'Connor et al. 1989; Paykel et al. 1994). All subjects included here had been prospectively clinically assessed with the CAMDEX interview (Roth et al. 1988). In cases where the last clinical assessment had taken place more than a year prior to death, a structured standardized proxy interview was made with a close relative or other informant to establish the patient's mental and physical state in the time prior to death. The diagnosis was made on the basis of the most recent CAMDEX interview. The CAMDEX criteria for a diagnosis of dementia are similar to those for DSM-III-R. In the majority of cases an interview was conducted with an informant after brain donation to confirm the clinical state of the patient in the period before death. This structured standardized interview was always undertaken when the last clinical assessment had taken place more than 1 year prior to death.

Brains obtained post mortem were cut in the sagittal plane. One cerebral hemisphere was dissected, macroscopically examined, and frozen. The other half of the brain was formalin-fixed for at least 3 weeks. For diagnostic purposes blocks for paraffin embedding were taken from the hippocampus at the level of the lateral geniculate body and from the entorhinal cortex at the level of the mammillary body; from the frontal, temporal, parietal, and occipital isocortex; from the basal ganglia, the thalamus, from two levels of the midbrain, from pons, medulla, and cerebellum.

Seven-micrometer-thick sections were cut and stained with H&E to assess nerve cell loss, gliosis, and ischemic change; and congo red to assess vascular amyloid deposits. The silver method of Campbell et al. (1987) and immunohistochemistry with a monoclonal antibody against β/A4 (donated by Dr. M. Landon, Department of Biochemistry, Nottingham University, UK) were used to demonstrate β-amyloid. A monoclonal antibody against phosphorylated tau-protein (11.57; Mukaetova-Ladinska et al. 1993) was used to label neurofibrillary tangles. The neuropathological assessment was performed blind to the clinical data.

Morphometry and staging procedure

Eleven laminae/areas were selected for the quantification of NFT for this study: the transentorhinal lamina pre-alpha, the entorhinal laminae pre-alpha, pre-β, and pri, the hippocampal area CA1, the frontal lobe (Brodmann area 9/10), the inferior parietal lobule (Brodmann area 39/40), the posterior temporal lobe including superior and middle temporal gyrus (Brodmann area 20/21), and from the parastriate and striate cortex (Brodmann areas 18 and 17). The quantification of neurofibrillary pathology in the neocortex was based on counting six randomly chosen fields per section next to a sulcus: two fields each at the crest, the side, and the base of the sulcus. The fields were chosen randomly using a low power dissection microscope in which neurofibrillary tangles could not be resolved, and marked for subsequent morphometry for which a standard transmission light microscope was used at × 100 magnification. In those instances where no tangles were detected in any of the randomly chosen areas, a further area representing the most affected field was included as an additional measure to calculate the mean. This had the effect of minimizing the possibility of failure to detect tangles when these were extremely sparse. Counts were undertaken with the help of an ocular grid covering a field of 0.9 ×

0.9 mm. All values were calculated per square millimeter. In addition to morphometric analysis, each case was graded independently by H.J.G. and J.H.X. using the staging criteria proposed by Braak and Braak (1991). Where there was disagreement, the cases were discussed in detail until agreement was achieved.

Statistical analysis

Standard non-parametric tests, Mann-Whitney U-test, and Kendall's tau, were applied in view of the non-normal distribution of the data. The statistical package SPSS for Windows (Microsoft Corp., Redmond, WA) was used without altering default settings.

Results

Clinicopathological description of cases

A total of 50 cases were initially selected on the basis of availability of brain tissues and appropriate clinical information. Of those, 8 were excluded because of clinical and neuropathological evidence of vascular dementia according to the criteria of Chui et al. (1992). The remaining 42 included no cases with neurodegenerative disease other than Alzheimer-type pathology. A total of 22 persons had been clinically demented according to CAMDEX criteria (Roth et al. 1988) prior to death. The mean age at death was 89.1 years (SD 3.7 years; range 81–98 years) with no significant difference between the demented and non-demented cases. There were more women than men (27 of 42 were female), but there was no gender difference with respect to presence or absence of dementia. There was no significant correlation between age and tangle count in any brain region.

The relationship between regional hierarchy and clinical dementia

Figure 1 shows the regional tangle counts according to presence or absence of clinical dementia as defined by CAMDEX criteria. The only region in which the number of neurofibrillary tangles was significantly higher in cases with dementia was the association neocortex (according to Braak's original proposal averaging data for temporal, parietal, and frontal regions (U-test; $z = -3.53$, $P = 0.004$). None of the differences in the allocortical regions were significant. In the present study a separate analysis of the temporal, parietal, and frontal regions was added. It showed that each of them showed significantly higher NFT counts in demented cases compared with non-demented cases (for temporal $z = -3.20$, $P = 0.001$; for parietal $z = -3.2$, $P = 0.001$; for frontal $z = -3.84$, $P = 0.0001$). Cases with dementia had significantly more regions affected than those without ($z = -2.63$, $P = 0.0086$).

Figure 1 also shows that the violations to the hierarchical order were more prominent in the cases with dementia than in those without. This is particularly evident with regard to area CA1 of the hippocampus, where the upper quartile values were higher than the corresponding values in the entorhinal cortex. In the non-demented cases the median value in CA1 was marginally higher than in lamina pre-alpha.

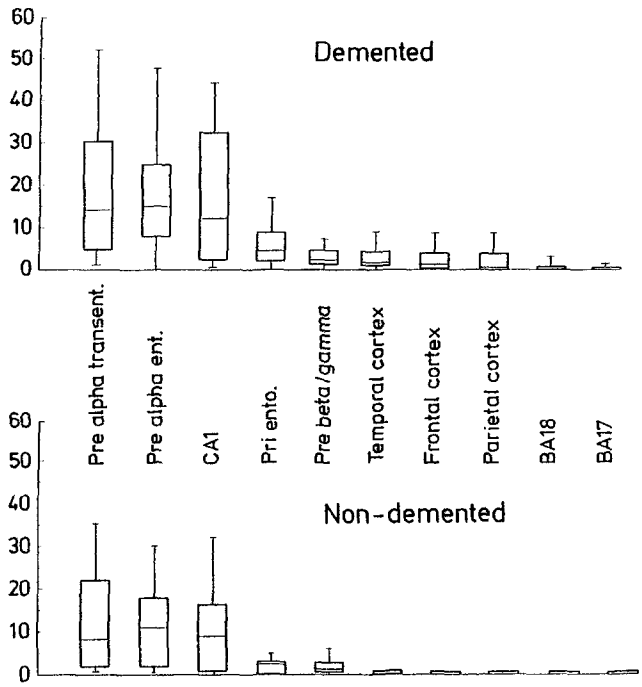

Fig. 1 Neurofibrillary tangle numbers of the different brain regions for demented ($n = 22$) and non-demented ($n = 20$) cases according to the CAMDEX diagnostic criteria. The *lower boundary* of the box is the 25th percentile and the *upper boundary* is the 75th percentile. The *horizontal line* inside the box represents the median

Table 1 Neuropathological stages for neurofibrillary tangle as a qualitative judgment according to Braak and Braak (1991) and the number of cases diagnosed as demented or non-demented per stage (numbers in parentheses are percentages)

Neuropathological stage	Non-demented ($n = 20$)	Demented ($n = 22$)
1	2 (10.0)	1 (4.5)
2	5 (25.0)	1 (4.5)
3	8 (40.0)	6 (27.3)
4	3 (15.0)	6 (27.3)
5	2 (10.0)	7 (31.8)
6	0	1 (4.5)

In order to determine whether the neuropathological staging system provides a means of defining a neuropathological threshold for the appearance of clinical dementia, the frequency of cases at each neuropathological stage was tabulated according to presence or absence of clinical dementia (Table 1). At stages 1–3 there were fewer cases with dementia than cases without (8 of 22 vs 15 of 20 respectively). At stages 4–6 there were more cases with dementia than without (14 of 22 vs 5 of 20). The transition from stage 3 to stage 4 was associated with a statistically significant difference in the likelihood of the presence or absence of clinical dementia ($\chi^2 = 6.31$, $P = 0.01$), but there was a large degree of overlap. At stage 4, 3 of 9 cases (33%) were not, whereas at stage 3, 6 of 14

cases (43%) were demented. Therefore, the transition from stage 3 to stage 4 is a statistical, not a diagnostic, threshold.

Discussion

Morphological studies of Alzheimer's disease in which the type and extent of neuropathological lesions in clinically demented subjects are compared with cognitively intact age-matched controls are likely to introduce artificial dichotomies by excluding borderline cases (Brayne 1993). Intermediate cases are potentially the most informative for understanding the neurobiology of transition from normal to impaired cognitive functioning in later life. This clinicopathological study overcomes this problem because it is based on a community sample defined in the course of prospective epidemiological studies on the cognitive decline of the aging population. The only inclusion criteria were those of the original epidemiological studies (O'Connor et al. 1989; Paykel et al. 1994), availability of brain tissues, and appropriate prospective clinical data. This approach made it possible to obtain cases concentrated at the mid-range of both cognitive decline and pathology. Only 1 of the 22 demented cases has been classified as severely demented according to the CAMDEX protocol. This excludes the possibility that our results are biased by a ceiling effect. In terms of pathology 21% are at stages 1 and 2, and 24% are at stages 5 and 6. The remaining 55% were at the intermediate stages 3 and 4. The exclusion of cases with vascular dementia according to the criteria of Chui et al. (1991) was required to permit correlation of neurofibrillay pathology with clinical data.

It is interesting to note that in this very elderly cohort (ninth and tenth decade of life) no case was found without neurofibrillary tangles. This is in agreement with other studies of cohorts of similar age (Gellerstedt 1933; Tomlinson et al. 1968; Matsuyama 1978, 1983). Much recent work has focused on the specific molecular substrates of Alzheimer's disease (Robakis 1994; Wischik et al. 1995), particularly potential genetic causes (St. George-Hyslop 1994). This study again reveals the basic importance of aging per se as an etiological factor. Tangles first appear in the middle years of life and increase steadily with aging (Ball 1976; Braak and Braak 1991).

The neuropathological staging tends to differentiate cases with and without dementia to some extent. Non-demented cases tend to have lower stages, whereas demented cases are found mainly in the more advanced stages. Because the majority of the cases examined were at intermediate neuropathological stages, this study is particularly helpful in determining whether the staging model taken as a whole identifies a neuropathological threshold for clinical dementia. Although the transition from stage 3 to stage 4 is associated in a statistically significant manner with the appearance of clinically diagnosed dementia, neuropathological stage 4 can occur without clinical dementia (33% of cases), and neuropathological stage 3 can occur with clinical dementia (43% of cases). Even in the group at neuropathological stage 5, 33% of cases did not reach clinical criteria for a diagnosis of dementia. Therefore, the neuropathological staging system reflects a broad statistical tendency for an increasing likelihood for dementia to be apparent clinically in the presence of both higher overall tangle counts and the involvement of more brain regions. In this context it is important that the transition from stage 3 to stage 4 is marked by the appearance of NFT in the association cortex, particularly in the temporal and frontal cortex. Of all single areas used in the staging system, the regions of the associated cortex are those regions in which NFT counts are statistically different in demented and nondemented cases. The threshold appearing in the neuropathological staging model seems to be due to the involvement of the temporal, frontal, and parietal cortex. Thus, the neuropathological staging system does not offer greater certainty in the neuropathological diagnosis of Alzheimer's disease than the well-established, but equally uncertain, criteria used by Tomlinson et al. (1976), who screened the temporal cortex for NFT.

The importance of specific architectonic areas of the allocortex in the neurophysiology of higher cortical function has become apparent in recent years (Van Hoesen et al. 1982; Treves and Rolls 1995). Multimodal corticocortical projection pathways converge on the neurons of lamina pre-alpha of the entorhinal cortex. These are the cells of origin of the perforant pathway and provide the main cortical input to the hippocampus. The pyramidal cell layer of CA1 and subiculum of the hippocampus provides the main hippocampal output back to the neocortex via projections to layer 4 of the entorhinal cortex. Although much has been made of the apparent ordering of neuronal degeneration at these three relay points, with particular emphasis given to lamina pre-alpha of the entorhinal cortex as the critical anatomical substrate of cognitive dysfunction (Hyman et al. 1984), our previous quantitative studies failed to provide support for any consistent ordering of pathology in this circuit (Gertz et al. 1995). The only reliable conclusion from our study is that all three relay points in this circuit are vulnerable to pathology. In our series there was extensive pathology in the allocortical laminae/regions in cases without evidence of clinical dementia. Furthermore, the extent of pathology in these regions did not distinguish statistically cases with and without clinical dementia as defiend by CAMDEX.

In conclusion, the extent of neurofibrillary pathology in the circuitry of the allocortex does not provide a quantitative basis for understanding the transition to dementia in late life. Paradoxically, this is due to the validity of the hierarchical model itself, because it carries with it the implication that progression of neurofibrillary pathology into neocortical regions is necessarily associated with advanced pathology in allocortex. It then becomes formally impossible to decide whether to attribute dementia to involvement of the neocortex, or to advanced allocortical pathology causing disconnection, or a combination of both. The valuable contribution of the neuropathological staging model proposed by Braak and Braak (1991) is that

it made this problem explicit so that it could be operationally analyzed.

Acknowledgements This work was supported in part by the Medical Research Council (UK) grants to C.M.W., C.R.H, J.H.X., E.S.P., F.A.H., and C.B. E.B.M.L. was supported by the Leopold Müller estate. C.H. was supported by the Newton Trust Trinity College. H.J.G. was supported in part by Bayer AG, Germany. The epidemiological component of the study was funded by grants from the Charles Wolfson Trust, the Medical Research Council, and the regional Health Authorities. We express our gratitude to A. O'Sullivan, to R. Hazelman for assistance with brain tissue donation, to R. Hills and J. Hurt for technical assistance, and to S. West, as well as I. Thies, for secretarial assistance.

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