Progression of Clinical Deterioration and Pathological Changes in Patients With Alzheimer Disease Evaluated at Biopsy and Autopsy

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Objectives: To quantify the progression of senile plaques, neurofibrillary tangles, cerebral amyloid angiopathy, and microglial activation in the cortex and white matter of patients with Alzheimer disease evaluated at both biopsy and subsequent autopsy and correlate these changes with the progression of neurologic impairment.

Setting: Academic referral center for patient with Alzheimer disease.

Patients: Four patients meeting the clinical criteria for Alzheimer disease, enrolled in a pilot study for the evaluation of response to intracerebroventricular administration of bethanechol chloride. The patients were followed up until death occurred and autopsy was performed.

Results: All 4 patients had progressive deterioration from the time of biopsy to autopsy (9-11 years). Pathological investigations showed a striking increase in the density of senile plaques and neurofibrillary tangles in 2 of 4 patients from biopsy to autopsy, and a significant increase in microglial activation in 1 of 4 cases. Severity of cerebral amyloid angiopathy varied significantly among patients, 1 of whom displayed striking amyloid deposition with associated subcortical white matter atrophy.

Conclusions: These unique data demonstrate that the progressive neurologic impairment in Alzheimer disease is accompanied by a significant increase in senile plaque and neurofibrillary tangle counts in the frontal cortex and, possibly in some patients, by increased microglial cell activation. Cerebral amyloid angiopathy was associated with significant white matter disease.

Arch Neurol. 1999;56:1254-1261
PATIENTS AND METHODS

SUBJECTS

The subjects described in this article were originally part of a pilot dose-response study of ICV infusion of bethanechol as previously described. All of the patients were men. Each had a thorough medical and neuropsychiatric evaluation prior to biopsy from a gyrus of the right prefrontal cortex during placement of the ICV catheter; the clinical diagnosis of AD was confirmed pathologically using the criteria outlined by Khachaturian. Autopsy examination was subsequently performed on 4 patients (patients 1, 3, 4, and 5 using the numerical designation of the Bethanechol Trial). Their characteristics are summarized in Table 1. The period between biopsy and autopsy was 9 years for patients 1 and 5 and 11 years for patients 3 and 4. At the initial neuropsychiatric evaluation prior to biopsy, patients 1 and 3 scored 18 on the Mini-Mental State Examination (MMSE) and were rated 1 on the Clinical Dementia Rating Scale (CDRS); patients 3 and 4 scored 27 on the MMSE and and were rated 0.5 on the CDRS. Clinically, they met the National Institute of Neurological Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association criteria for a diagnosis of probable AD and Diagnostic and Statistical Manual of Mental Disorders, Third Edition criteria for primary degenerative dementia.

TISSUE PROCESSING

Extensive articles have already been published on the biopsy findings of all patients in the Bethanechol Trial, including a detailed description of tissue processing and immunohistochemical procedures. Biopsy and autopsy materials were fixed in 10% buffered formalin. After 1-cm-thick sections of brain had been obtained at autopsy, a sample of prefrontal cortex was removed in the immediate vicinity of the biopsy site, but without including the biopsy scar. The brains were also processed for routine histology. Blocks were taken bilaterally from the frontal, temporal, parietal, and occipital cortex, basal ganglia, hippocampus, diencephalon, and brainstem. Sections were stained using a modified Bielschowsky technique and hematoxylin and eosin; also, immunohistochemistry for Aβ amyloid and CD68 was carried out using standard techniques, as previously described.

ASSESSMENT OF DENSITIES OF SPs AND NFTs

Counts of NFTs and SPs were performed on both biopsy and autopsy specimens by 2 investigators (P.L.D.P. and L.M.V.), without knowledge of which autopsy specimen matched which biopsy specimen. Using a graticule, counting of SPs and NFTs was performed at magnification ×200 on Bielschowsky-stained sections and the counts were expressed per square millimeter of tissue. In both biopsy and autopsy specimens, 5 to 6 cortical areas spanning the whole cortical thickness were selected so as to cover the entire depth of the cortex in the selected areas. Both sulcal depths and crests of gyri were included in these assessments in roughly equal numbers. The interobserver variability in SP and NFT counts was within a 5% to 10% range.

EVALUATION OF AMYLOID IMMUNOHISTOCHEMISTRY AND MICROGLIAL DENSITY

Biopsy and autopsy sections immunostained with antibodies to Aβ and CD68 were semiquantitatively evaluated for the presence of intraparenchymal and vascular β-amyloid deposition and microglial density. The frontal cortex from the autopsy of 2 age-matched subjects with no history of neurologic disease and 2 patients with AD (autopsy tissue only available) were used for comparison with the cases in this study. Density of CD68+ microglia were graded as “low,” “moderate,” or “high.” Ratings were based on consensus observations.

ASSESSMENT OF APOE GENOTYPE

Genomic DNA was extracted from the paraffin sections of the brains available at autopsy, according to a procedure described previously. Briefly, paraffin sections were treated with xylene to dissolve the paraffin. The tissue was then centrifuged, treated with 100% ethanol, and digested with proteinase K (final concentration, 200 mg/mL) in a buffer containing 50 mmol/L Tris (pH 8.5), 1 mmol/L EDTA, 0.3% to 1.0% sodium dodecyl sulfate, and 0.1 mmol/L sodium chloride at 95°C for 3 hours. Proteinase K was inactivated by heating the tubes for 30 minutes at 95°C. The supernatant was treated with 5 mol/L of potassium acetate and subsequently with 7.5 mol/L ammonium acetate and 100% ethanol for at least 2 hours at –80°C. The DNA pellet was resuspended in Tris-EDTA (pH 7.8), and absorbance was measured in a spectrophotometer of 260 and 280 nm. Polymerase chain reaction amplification and APOE-restriction isotyping were carried out following the method of Hixson and Vernier.

STATISTICAL ANALYSIS

A 2-tailed t test was used to assess the significance of differences in the densities of NFTs and SPs between biopsy and autopsy in each patient. The average numbers of plaque and tangle counts in the available fields were used for statistical analysis. Also, to evaluate the effect of time on the changes of SPs and NFTs in all patients as a group, analysis of variance was used.

who originally were enrolled in a therapeutic trial to test the effects of intracerebroventricular (ICV) administration of the cholinergic agonist bethanechol. Senile plaques and NFTs were assessed in a biopsy specimen taken from the right frontoal cortex and, subsequently, in adjacent areas of the cortex examined at autopsy 9 to 11 years after the biopsy procedure. Also, the degree of cerebral amyloid angiopathy and microglial cell density were studied by immunohistochemistry for Aβ amyloid and CD68 (a marker for microglia and histiocytes). The progression of these changes will be described in conjunction with the time course of the cognitive and functional decline.
### Table 1. Patients’ Clinical Parameters

<table>
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<th>1</th>
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<th>4</th>
<th>5</th>
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<td>At biopsy</td>
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<td>11</td>
<td>9</td>
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<td>0</td>
<td>0</td>
</tr>
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<td>CDRS at biopsy</td>
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</table>

*AD indicates Alzheimer disease; MMSE, Mini-Mental State Examination; and CDRS, Clinical Dementia Rating Scale.

#### RESULTS

### PROGRESSION OF COGNITIVE AND FUNCTIONAL DETERIORATION

At enrollment and biopsy, all 4 patients had retired but were living at home with a spouse, with some degree of independence in activities of daily living. Detailed neuropsychologic testing (S.L.R., unpublished data) confirmed cognitive deficits in short- and long-term memory, language, ability to copy drawings, problem solving, and sequential thinking. As a benchmark, enrollment MMSE was 27 for patients 3 and 4 and 18 for patients 1 and 5 (Table 1). Postoperative complications included meningitis and seizures in patient 1, hemorrhage with mild paraparesis and seizures in patient 5. Patients 3 and 4 had uncomplicated postoperative courses.

Assessment of the immediate (2-year) postoperative period demonstrated a differential response to ICV bethanechol. Patients 3 and 5 had a positive response that was confirmed by a second trial of dose variation. These patients were able to return home for 4 and 5 years, respectively, before long-term care was required. Patients 1 and 4 had poor overall response dominated by depression due to bethanechol.

By 2 years after operation, the dementia had resumed a monotonic progressive course in all patients that continued relentlessly until death. All required nursing home care in their last years. Mini-Mental State Examination declined to zero prior to death in all 4 patients and all were dependent for personal activities of daily living. Although duration of postoperative survival was similar, the rate of decline was more rapid in patients 3 and 4 compared with patients 1 and 5.

### GROSS PATHOLOGY AT AUTOPSY

In all the patients’ brains, no evidence of acute intracranial hemorrhage, traumatic injury, or meningeal inflammation was identified. Atherosclerotic changes of branches of the circle of Willis were insignificant. No recent or remote infarcts were found, the only regions of encephalomalacia being the biopsy site. The substantia nigra and locus ceruleus were normally pigmented.

In patient 1, evidence of mild cortical atrophy was present, most prominent in the frontal lobes, hippocampi, and parahippocampal gyri. On coronal sections, a catheter tract was seen at 3 cm from the frontal pole, surrounded by an area of encephalomalacia of gray and white matter (Figure 1, A). The brain of patient 3 was also affected by extremely advanced cortical atrophy. The tip of the indwelling catheter was surrounded by a cavitary lesion measuring 2 cm in greatest diameter. Sampling of the cortex for SP and NFT counting was performed as close as possible to the site of the catheter but at a sufficient distance to exclude the area of gliosis surrounding the biopsy cavity. The brain of patient 4 displayed the most advanced degree of cerebral atrophy, associated with striking ventricular dilatation (Figure 1, B) and shrinkage of the subcortical white matter. In this brain, the catheter had previously been removed, and no evidence of a catheter track could be appreciated on gross or microscopic examination. In patient 5, severe diffuse cortical atrophy was present, with extreme narrowing of the gyri, approaching the appearance of “knife-edge atrophy” in some regions. An indwelling catheter was identified, with surrounding mild encephalomalacia and yellow-orange discoloration, suggestive of blood pigment deposition (Figure 1, C).

### DENSITY OF SPs

Figure 2 shows the densities of SPs at biopsy and autopsy for all cases. We found a marked, statistically significant increase of SP density in patients 3, 4, and 5, with counts at autopsy being 2.5, 3.0, and 8.4 times, respectively, those at biopsy. In patient 1, no significant change in SP density was detected. The increase in SP counts appeared to result from an increase in all types of plaques, including diffuse, neuritic, and amyloid plaques with no recognizable predominance for any of these subtypes (for nomenclature and definition of plaque types, see Dickson). Specifically, neuritic plaques and diffuse plaques were present in approximately equal numbers (50% each type), and this ratio did not vary significantly from biopsy to autopsy. No preferential laminar involvement was appreciated in changes of SP densities. When all cases...
were considered together, analysis of variance showed a statistically significant difference between SP counts at biopsy and those at autopsy.

**DENSITY OF NFTs**

As indicated in Figure 3, NFT counts showed, from biopsy to autopsy, a marked, statistically significant increase in patients 1 and 5, a mild increment (close to statistical significance, \(P = .05\)) in patient 3, and an apparent decrease (not reaching statistical significance) in patient 4. The autopsy NFT densities increased 5.5, 1.6, and 4.7 times in patients 1, 3, and 5, respectively, and decreased by a factor of 0.7 in patient 3. The increased numbers of NFTs were attributable to a uniform increase in density throughout all layers, with no apparent preferential laminar involvement. Analysis of variance indicated a statistically significant difference between biopsy and autopsy NFT counts when all cases were considered together.

**TOPOGRAPHY OF SPs AND NFTs AT AUTOPSY**

**Figure 4** shows the relative densities of SPs and NFTs in different neocortical regions of 2 patients assessed at autopsy (1 and 5). A remarkable topographic variability in the distribution of these lesions is evident.

**IMMUNOSTAINING FOR A\(\beta\) AMYLOID AND CD68**

As previously reported, biopsy tissue displayed varying amounts of amyloid deposition within intraparenchymal and leptomeningeal vessel walls.\(^{17,18}\) Patient 4, in particular, displayed a severe degree of cerebral amyloid angiopathy, which was especially prominent in cerebral arteries and arterioles, but marked within capillaries as well (Figure 5, A). Autopsy sections of patient 4 revealed amounts of vascular amyloid comparable with those in the biopsy specimen, without apparent changes in extent or pattern of amyloid deposition. The white matter displayed marked thinning and pallor, due to loss of axons and myelin, with concomitant widespread astrogliosis (Figure 5, B). In all other cases, modest amyloid deposition was demonstrated in the microvasculature of both biopsy and autopsy sections, with no appreciable changes from biopsy to autopsy.

Similarly, CD68 immunoreactive microglial cells were present in both biopsy and autopsy material in variable densities. **Table 2** shows the results of a semiquantitative assessment of microglial density in the biopsy and autopsy samples, subjectively graded as low, moderate,
or high, in the cortex and subcortical white matter. In general, the density of microglial cells in autopsy material was approximately similar to that seen in the corresponding biopsy material, except in patient 5, where microglial density increased significantly from biopsy to autopsy (Figure 6), in both gray and white matter. Comparing white matter with gray matter within the same case, we observed that microglial cell densities were consistently much higher in the white matter than in the cortex, both at biopsy and at autopsy. Microglial cells showed a monotonous and even distribution throughout the white matter, while in the neocortex they were diffusely dispersed, but with occasional focal clustering (presumably around senile plaques). Frontal lobe samples derived from the autopsy of 2 neurologically intact subjects showed low microglial densities in both gray and white matter, with only rare microglial cells showing scanty cytoplasm and short processes. The frontal cortex and white matter of 2 controls with AD had moderately increased microglial densities that were approximately comparable with those of the cases in our study. However, microglial cell density in patient 5 was noticeably higher than all other AD cases (Figure 6).

**APOE AND PRESENILIN GENOTYPING**

APOE genotypes could be determined on patients 1 and 3 only. Both these cases had a 3/4 APOE genotype. Also, a blood sample from patient 3 was analyzed by the Genetics Project of the University of Washington Alzheimer’s Disease Research Center, Seattle (T. Bird, MD, G. Schellenberg, MD, 1998) and revealed a missense mutation in the presenilin-1 gene (R269H).

**COMMENT**

The present investigation was aimed at studying the progression of AD changes, eg, SPs and NFTs, in 4 patients whose brains were examined at autopsy and who had previously undergone a biopsy procedure 9 to 11 years before death. These patients afforded the rare opportunity to conduct a longitudinal study to help clarify the still obscure issue of the temporal evolution of pathological changes in AD. All our patients showed a consistent and marked decline in their cognitive functions throughout the period between biopsy and autopsy, as indicated by their functional ability and MMSE scores, which declined from 18 (in patients 1 and 5) and 27 (for patients 3 and 4) to 0 by the time they died. Concomitantly, a marked, statistically significant increment in the density of SPs in the right frontal cortex was revealed, with counts at autopsy reaching levels from 2.5 up to 8.4 times those recorded in the biopsy specimens for 3 of the patients (patients 3, 4, and 5), while they remained unaltered in patient 1. Similarly, autopsy NFT densities showed a marked statistically significant increase in patients 1, 3, and 5 (1.6-5.5 times those at biopsy); in patient 4, the autopsy NFT counts were lower than in the biopsy but not to a statistically significant degree. No apparent correlation was noted between the presence of a family history (in patients 2 and 5) and evolution of Alzheimer changes from biopsy to autopsy. Tissue shrinkage due to brain atrophy may have contributed to an apparent increase in NFT and SP densities but not to the extent observed in this study. The effect of catheter insertion seems unlikely to have played a role in producing or accelerating progression of AD changes, but no data are available in the literature to demonstrate that focal trauma may have an effect on SP and NFT development at sites distant from the lesion itself.

Clinicopathologic investigations have suggested that the severity of the intellectual impairment in patients with AD is correlated with the burden of underlying pathological
Figure 6. Photomicrographs of sections of biopsy and autopsy frontal tissue from patient 5, immunostained for CD68. Included as controls are photomicrographs of CD68-immunostained section of frontal white matter from a neurologically intact elderly person and a patient with Alzheimer disease. Pictures were taken at the original magnifications given in parentheses. A, Patient 5, biopsy, cortex (×200); B, patient 5, biopsy, white matter (×200); C, patient 5, autopsy, cortex (×200); D, patient 5, autopsy, white matter (×200); E, patient 5, autopsy, white matter (×400); F, Alzheimer patient control, frontal white matter (×400); G, neurologically intact elderly control, frontal white matter (×400). Note marked increase in density of CD68-positive microglial cells in both cortex (C, arrows), in contrast to their diffuse and even distribution in the white matter. Also, increased microglial density is evident in white matter of patient 5 compared not only with normal control (in which rare small microglial cells are seen) but also with Alzheimer disease autopsy control.
markers, ie, SPs and NFTs. If this is true, worsening of memory function in an individual patient should be accompanied by a concomitant increase in the numbers of SPs and NFTs. Such a view is in good agreement with the results of the present study, which shows that 4 patients with AD displayed a marked progression in the severity of neuropathological changes during the 9 to 11 years between biopsy and autopsy. Two previous studies, apparently similar to ours in design and patients, reached different conclusions, as no significant progression of AD changes was seen in patients who showed progressive mental decline in the period from biopsy to autopsy. This discrepancy can probably be explained by examining in more detail the individual patients investigated in those studies. In the investigation by Mann et al., the study group consisted of 5 patients, of whom 2 had frontal and 3 temporal biopsies. Available data indicate that there are marked interindividual differences in the regional brain distribution of AD pathology, as well as in the apparent progression of lesions in the frontal vs temporal lobes. It seems appropriate, therefore, to compare our patients (whose pathological analysis was carried out in the frontal cortex) exclusively with the subjects in whom biopsy and autopsy data were obtained from the frontal cortex. In these patients, the time interval between biopsy and autopsy was only 3 years, as compared with 9 to 11 years in our cases. Similarly, the study by Bennett et al. examined SP and NFT densities in the frontal cortex of patients who were followed up for only 21 to 47 months from biopsy to autopsy. The difference in length of follow-up seems the most probable reason for the discrepant results between our study and those of Mann and colleagues and Bennett et al. In addition, the relatively short period from biopsy to death in the patients of the previous studies suggests that they underwent biopsy at more advanced stages of disease compared with our patients. It is plausible that in the most advanced stages of AD a “ceiling effect” may occur, when intellectual deterioration begins to gradually decelerate and the underlying pathological changes also cease to progress. The patient in the study by Bennett and colleagues who was followed up for the longest time (47 months) manifested a statistically significant increase in the density of NFTs. Thus, the groups of patients presented by the other investigators may have failed to show any progression of pathological changes possibly because they were examined in the “end stage” of the disease, in which no further numerical increment of SP and NFT densities become apparent. Discrepancies between degree of neurologic impairment and counts of associated SPs and NFTs may also have other explanations.

Pathological alterations other than SPs and NFTs have been shown to develop in AD brains and parallel the severity of the clinical picture. At least 3 additional pathophysiologic processes have been proposed to play a key role in the pathogenesis of AD: (1) Loss of synapses in AD frontal cortex has been well documented by synaptophysin immunoreactivity and ultrastructural analysis and shown to be correlated with the degree of cognitive deficit. (2) Inflammatory mechanisms and microglial cell activation may also participate in the pathophysiology of AD and possibly exert detrimental effects clinically. Microglial cell density evaluated in our samples by CD68 immunohistochemistry shows that activation of microglial cells varies significantly from subject to subject and may also manifest a considerable increase with time (such as in patient 5, increasing from “low” levels at biopsy to “high” levels at autopsy). Surprisingly, microglial cell density in the white matter was remarkably higher than that in the neocortex in all cases, suggesting that inflammatory responses involving the white matter may be more prominent than those in the gray matter and obviously not directly related to the development of the pathological features considered to be most characteristic of AD. (3) Cerebral amyloid angiopathy (CAA) is an additional, fundamental pathologic process of AD, which may contribute to brain damage by mechanisms not yet entirely clarified, but possibly including ischemia. With respect to degree of CAA, the present cases displayed a remarkable heterogeneity, with patient 4 showing the most severe and widespread vascular deposition of amyloid and also the most striking degree of brain atrophy (especially white matter) by the time of death. Previous investigations performed on the same subjects showed that the levels of amyloid precursor protein in the cerebrospinal fluid were negatively correlated with the burden of amyloid deposition within brain, being 20 times lower in patient 4 (with the most severe CAA) than in the remaining 3 patients. Other investigators have reported similar findings. These data suggest that levels of soluble Aβ amyloid and amyloid precursor protein are inversely correlated with amyloid burden in the cerebral vessels and may be used as a useful marker for diagnostic purposes and as a measurement of clinical progression of AD.

Alzheimer disease pathological markers develop in vulnerable brain regions in an uneven manner and at a variable rate. Indeed, cross-sectional studies indicate that the spreading of pathological alterations in AD does not appear in different brain regions at the same time but tends to begin in certain areas (namely, entorhinal cortex and hippocampal formation), subsequently spreading to other areas as the disease advances. Obviously, counting SPs and NFTs in a small sample of frontal cortex may not be sufficient to account for the progression of cognitive deterioration.

Finally, progression of cognitive impairment may not be exclusively correlated with increasing SP and NFT densities or with numerical changes of other variables but may also depend on the specific brain regions affected. Involvement of subcortical nuclei crucial to memory functions, such as cholinergic forebrain nuclei, contributes importantly to cognitive impairment. These nonplaque or tangle changes may help to account for the absence of any increase of SP density in patient 1 and of NFT density in patient 4 of our study, despite increasing cognitive impairment.

In conclusion, although these patients (who had early-onset AD) may not be representative of all, and especially late-onset, patients with AD, these data are consistent with the prevalent view that clinical progression and severity of AD is paralleled by, and probably correlated with, progression of the underlying pathological changes, ie, SPs and NFTs in the neocortex. Furthermore, the presence of severe CAA in one case and of marked microglial cell activation in another reemphasize the notion that the underlying pathology of AD may be heterogeneous: in addition to SP and NFT forma-
tion, microglial cell activation and CAA may be additional pathogenetic factors whose role in the progression of AD neurologic impairment is important but still not clearly defined. It is also apparent that the relationship between progression of pathologic changes and clinical deterioration is rather loose, possibly because neither SP nor NFTs represent the brain fundamental morphologic change underlying AD. Based on the available data, therefore, it is plausible that AD may result from a mosaic of pathologic changes that vary from patient to patient in intensity and distribution, and whose variability may account, ultimately, for the dissimilarities in clinical picture seen among patients with this disease.

Accepted for publication September 10, 1998.

This work was supported by US Public Health Service, Washington, DC, grants PO1 NS 12435, P30 AG 10123, and the Sidell-Kagan Foundation, Los Angeles, Calif. The original Bethanechol Trial was supported by the John Douglas French Foundation for Alzheimer’s Research and the Veterans Affairs Medical Center, West Los Angeles.

The views expressed do not necessarily reflect the views of the Veterans Affairs or the US government.

Carol Appleton prepared the illustrations; Zhen Zhen Wang, MD, assisted with immunohistochemical study of CAA; and Lynn A. Fairbanks, PhD, assisted with the statistical analysis. APOE genotyping was carried out by the Alzheimer’s Disease Center Genetics Core of University of California, Los Angeles.

This study would not have been possible without the extraordinary commitment of the Bethanechol Trial patients and, especially, their wives and families.

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REFERENCES