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Neuropathological Criteria for the Diagnosis of Alzheimer's Disease

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MARKESBERY, W. R. *Neuropathological criteria for the diagnosis of Alzheimer's disease.* NEUROBIOL AGING **18**(S4), S13–S19, 1997.—The definitive diagnosis of Alzheimer's disease (AD) is made at autopsy by the presence of abundant neuritic plaques (NP) and neurofibrillary tangles (NFT) in the neocortex, entorhinal cortex, and hippocampus. The two criteria most frequently used by neuropathologists for the diagnosis of AD over the past 12 years are those described by Khachaturian and the Consortium to Establish a Registry for Alzheimer's Disease. Though both have been useful, they have weaknesses and lack validation. The majority of recent studies has shown that NFT in the entorhinal cortex, hippocampus, and neocortex and NP in the neocortex correlate best with severity of dementia in AD. The criteria recommended by the Workshop on Diagnostic Criteria for the Neuropathological Assessment of AD uses semiquantitation of NFT and NP in the neocortex, adds evaluation of the hippocampus and entorhinal cortex, places emphasis on coexisting lesions such as vascular lesions and Lewy bodies, and establishes criteria for general pathologists and more rigorous criteria for the AD research setting. These criteria will require further refinement and validation. © 1997 Elsevier Science Inc.

Alzheimer's disease Neuropathological criteria Neurofibrillary tangles Neuritic plaques Normal aging Neocortex Entorhinal cortex Hippocampus

TRADITIONALLY, the definitive autopsy diagnosis of Alzheimer's disease (AD) has been made by neuropathologists in patients with a clinical history of dementia when abundant senile plaques (SP) and neurofibrillary tangles (NFT) are present in the neocortex, entorhinal cortex, hippocampus, and amygdala. There is considerable subjectivity, variability, and inconsistency by neuropathologists in determining the diagnosis of AD. As interest in AD increased in the 1960–1980 period, it became obvious that definitive criteria for the histopathological diagnosis of AD were needed.

In 1984, a group of distinguished neuropathologists recommended minimal microscopic criteria for the diagnosis of AD as described by Khachaturian (33). These were intended to serve as guidelines to aid in development of uniform procedures rather than representing validated methods and quantitative threshold values. They suggested reviewing sections of three neocortical regions (frontal, temporal, and parietal), amygdala, hippocampus, basal ganglia, cerebellum, and spinal cord. They also suggested that the section should be 5 to 15 microns thick and stained with the Bielschowsky method, congo-red, or thioflavin S. After excluding other causes for dementia, they recommended using the histopathologic criteria for the diagnosis of AD that is shown in Table 1.

These criteria have been used by many neuropathologists for years, and they have served a useful function by focusing efforts on refining and improving the diagnosis of AD. They have yet to be validated uniformly.

Several problems exist with these criteria: a) The type of SP is not specified. The brains of many nondemented elderly contain sufficient diffuse plaques to meet the diagnostic criteria for AD (discussed below). Neuritic plaques (NP) are more closely correlated with the severity of AD; b) Precise quantitation of SP is difficult, especially when defining the discrete margins of SP and counting tiny amyloid deposits. Determining the amyloid beta-peptide (Aβ) burden may be a more accurate method of defining the degree of amyloid deposition; c) The presence of NFT were required only for cases under 50 years old; d) The criteria apply to changes in the neocortex alone and do not take into consideration the entorhinal cortex, hippocampus or amygdala, where striking changes are found, and; e) The relationship of the neuropathological findings to the clinical history is vague.

In 1988, Tierney et al. (55) attempted to validate the criteria described by Khachaturian (33) against differing neuropathology criteria for AD. They used three sets of criteria with one or more NFT and NP per 25 × microscopic field in: a) hippocampus alone, b) both neocortex and hippocampus, and c) neocortex alone. They also used three sets of vascular disease exclusion criteria. They found that the accuracy of all three sets of criteria ranged from 81% to 88%. The most accurate was the presence of NFT and NP in the hippocampus alone. In three of their cases, these lesions were present in the hippocampus, but not in the neocortex. The criteria used in this study have not been adopted widely.

In 1991, the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) proposed standardized neuropathological criteria for the postmortem diagnosis of AD (43). The criteria were designed to create a data base for many potential uses including refinement of diagnostic criteria. The criteria are based on the

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TABLE 1
HISTOPATHOLOGIC CRITERIA FOR THE DIAGNOSIS OF AD

Patient Age	SP in neocortex per 200× field	NFT in neocortex per 200× field
<50	2–5	2–5
50–65	8 or more	some
66–75	10 or more	some
>75	greater than 15	may or not be present

In the presence of a positive clinical history of AD, the criteria should be revised downward.

semiquantitation of NP by silver or thioflavin S stains in areas of maximum density in three neocortical regions: middle frontal gyrus, superior and middle temporal gyri, and inferior parietal lobule. An age-related NP score is determined on the basis of sparse, moderate, or frequent plaques in individuals in three age categories: less than 50, 50 to 75, and over 75. This score is then integrated with clinical information regarding the presence or absence of dementia to yield the level of certainty of the diagnosis of AD, i.e., definite, probable, or possible AD. This approach is desirable in that it uses a standardized method that is uncomplicated, and can be used by practicing pathologists without extensive neuropathology training or experience. It also uses the presence or absence of a clinical history of dementia to achieve a diagnostic level of certainty. These criteria have been used widely and have helped establish a common vocabulary among pathologists.

The CERAD criteria have several weaknesses: a) NFT, which are important in the diagnosis of AD, are not used; b) hippocampus, entorhinal cortex, and amygdala are not used for the diagnosis; c) although the semiquantitative approach is described clearly and demonstrated by cartoons, a degree of subjectivity still exists; and d) NP alone are used for diagnosis, although they exhibit a great degree of variability and density, and they have an uneven distribution in the neocortex.

Braak and Braak (10,11) described criteria for the pathological staging of AD into six stages based on the distribution pattern of neurofibrillary change (NFT and neuropil threads). These authors describe selective involvement of specific architectonic units that follow a predictable sequence during the course of the disease starting in the entorhinal cortex and spreading to the hippocampus and eventually the neocortex. In stages I and II (transentorhinal stage), NFT and neuropil threads are present in the transentorhinal layer pre-alpha (stage I) and CA1 of the hippocampus (stage II). Stages III and IV (limbic stage) are characterized by severe involvement of layer pre-alpha with spread to the hippocampus and basolateral amygdala. Stage IV shows severe transentorhinal and entorhinal involvement along with the amygdala and limbic nuclei of the thalamus. Stages V and VI (isocortical stage) are characterized by a spread to the basal portion of the isocortex and subsequent spread to the superior portions in the associational areas. The key feature is the severe destruction of neocortical association areas. Only stages V and VI meet the criteria described above for the neuropathological diagnosis of AD. This approach does not place emphasis on NP, and the quantitation of neurofibrillary pathology is not specified. These criteria need to be validated by clinical–pathological correlation with longitudinally followed patients. Recently, we evaluated 59 subjects from the Nun Study to determine if the Braak stages are associated with cognitive performance (21). Transition from the transentorhinal stages to the limbic stages was associated with a small but significant ($p < 0.05$) decline in performance on memory tests, but there was no change in performance on other cognitive tests. Transition from the limbic stages to the isocortical stages was associated with significant declines on all cognitive tests. This suggests that neurofibrillary pathology in the limbic region are weakly associated with a decline in memory, whereas neurofibrillary pathology in the neocortex is strongly associated with a global cognitive decline characteristic of AD.

There is considerable disagreement about the relative importance of NFT and SP in the neuropathological diagnosis of AD. A significant positive correlation between neocortical SP density and severity of dementia has been demonstrated (6,18). Similar positive correlations between NFT density or neuritic pathology and dementia severity have been described (2,4,5,18,40,44). Arriagada et al. (2) found the severity of dementia correlated with the number of NFT in the neocortex but not the degree of SP formation. They described accumulation in a consistent pattern reflecting hierarchic vulnerability of individual cytoarchitectural fields and that NFT appeared in the entorhinal cortex, CA1/subiculum, and amygdala early in the disease. Bierer et al. (5) found that only neocortical NFT correlated with Clinical Dementia Rating Scale scores in AD and most strongly correlated with NFT in the superior temporal, inferior parietal, and middle frontal regions. No statistically significant correlations were present for NFT in the amygdala, hippocampus, or entorhinal cortex, or for SP density.

Berg et al. (4) reported the following results of autopsy studies of 37 prospectively evaluated AD and 5 control subjects over 80 years of age: a) nondemented individuals had few or no neocortical SP or NFT, whereas all AD subjects had abundant neocortical SP; b) neocortical NFT densities but not SP densities correlated with dementia severity and duration; and c) hippocampal NFT densities were high even in subjects with mild AD, supporting the criteria for stages I and II of Braak and Braak (10,11).

Kazee et al. (32) found that mean NFT and NP counts discriminated AD from controls in a study of 42 pure AD and 17 nondemented controls who had been longitudinally evaluated prior to death. Abundant diffuse plaques and NFT in the neocortex were observed only in AD cases. They found that neocortical NFT were not an absolute requisite for the diagnosis of AD because a subset of AD patients in their study did not have neocortical NFT. They concluded that neither cognitive impairment nor neocortical NP or hippocampal NFT alone was sufficient for the diagnosis of AD. Nagy et al. (47) demonstrated a strong correlation between cognitive deficit and density of NFT in frontal and parietal lobes. Neurofibrillary tangle density also correlated with NP density.

Recently, Cummings et al. (14,15) quantified SP, NP, NFT, dystrophic neurites, and Aβ load and PHF-1 load by computer-based image analysis in relationship to cognitive ability in the entorhinal cortex of 16 demented and 4 control subjects. They found that all pathologic measures correlated with the severity of dementia, however, the strongest predictor of cognitive dysfunction with Aβ load. Their report included only one brain region and only four control subjects with all lacking premortem neuropsychological testing. These are limiting factors in this study.

The role of the diffuse plaque in AD is controversial. Diffuse plaques are thought to be the forerunner of the NP by some. It has been suggested that SP begin as extracellular, nonfilamentous, Aβ deposits that induce neuritic degeneration following fibrillogenesis (16). Others have suggested that all diffuse plaques have dystrophic neurites (50). Some studies have shown that diffuse plaques are commonly found in brains of nondemented elderly (see below). Kazee et al. (32) indicated that abundant neocortical diffuse plaques and NFT were observed only in AD cases. Others have indicated that diffuse plaques are the most common type found in AD (30).

In summary, most recent studies indicate that NFT are impor-

tant in the diagnosis of AD. The accumulation of NFT in the entorhinal cortex, hippocampus, and amygdala is not a good indicator of AD because they accumulate in these regions in both normal aging and AD. In some subjects, their presence in these regions may correlate with mild to moderate memory decline and represent the earliest phase of AD. However, the presence of significant numbers of NFT in the neocortex is associated with global cognitive decline and thus, they are a good marker for the diagnosis of AD. It appears diffuse plaques alone have yet to be convincingly demonstrated to correlate with the intellectual decline in AD. However, it is clear that neocortical NP correlate with the intellectual decline in AD. This leads to the conclusion that it is the presence of both neocortical NFT and NP that are important for the histopathological diagnosis of AD.

HISTOPATHOLOGIC FINDINGS IN NORMAL AGING

Distinguishing the histopathologic alterations in AD from the changes associated with normal aging in some instances can be difficult. Multiple autopsy studies have demonstrated clearly that NFT are present in the entorhinal cortex, CA1/subiculum, and amygdala, and SP are present in the neocortex and hippocampus of nondemented subjects (1,7,12,23,31,45,48,49,52). Although the regional prevalence and density of SP and NFT are quite variable, their distributions are prominent in regions that are most involved in AD. Even though pathological studies in persons over the age of 55 years suggest the presence of at least a few SP and NFT (1) and that SP may increase with advancing age (23), other data suggest the accumulation of SP is not inevitably related to normal aging (32,38).

Only modest numbers of autopsy studies of normal elderly have used subjects who had prospective neuropsychological testing (4,7,31,44,45,52). In our autopsy study (52) of 18 longitudinally-followed, normal, older adult volunteers, we found that the brains of 17% had essentially no histopathological alterations, and 44% had sufficient neocortical SP to meet the criteria described by Khachaturian (33) for the diagnosis of AD. There were no differences in a battery of 14 mental status tests between those meeting AD criteria and those who did not. Those who met the criteria had statistically significant greater numbers of both NP and diffuse plaques in four neocortical regions than those who did not. Mean neocortical NFT counts were not significantly different in the two groups, although small numbers of NFT were present in the middle temporal gyrus ($2.39/.586mm^2$) and occipital area 18/19 ($3.79/.586mm^2$) in the AD-like group. Parahippocampal gyrus mean NFT counts were significantly higher in the AD-like group. It is possible that the AD-like group was in the preclinical or incipient stage of AD, and that the battery of mental status tests was not sensitive enough to detect early cognitive dysfunction in this highly educated group.

Katzman et al. (31) described 10 prospectively evaluated subjects with preserved mental status and few or no cortical NFT but with marked numbers of neocortical NP. In another group of 19 nondemented elderly, there were few or no cortical NP and no NFT. Both groups had similar small numbers of hippocampal NFT. They suggested that the former group had incipient AD. Kazee et al. (32) also described a subgroup of cognitively normal controls who had neocortical NP.

Bouras et al. (7) showed that nondemented aged individuals frequently have NFT in the entorhinal cortex and hippocampus but not in temporal neocortex. Only demented patients had neocortical NFT. Amyloid deposits were not correlated with the clinical diagnosis and were present in neocortex earlier than in the hippocampus. They suggested that NFT in the hippocampus may be a necessary, but not sufficient, condition for the clinical expression of dementia that is more closely related to neocortical NFT.

Morris et al. (45) described neuropathological findings of 21 longitudinally studied healthy elderly subjects. Nine of the subjects had high SP densities in the neocortex and seven of these had Clinical Dementia Rating Scale scores of 0.5 and mildly impaired psychometric performances at the last assessment before death. The SP were predominantly the diffuse type, but small numbers of NP were present. They met the histopathological requirement for AD as described by Khachaturian (33). These individuals also had hippocampal and entorhinal NFT. They suggested that SP may not be present in normal aging but instead represent presymptomatic or incipient AD. However, the other 12 subjects in their study had small numbers of SP, raising the question of whether SP density is an important factor in dementia.

In summary, most studies confirm that modest numbers of NFT and diffuse SP are common in nondemented elderly subjects. Neurofibrillary tangles are most prominent in layer 2 of the entorhinal cortex, CA1/subiculum, and amygdala in nondemented elderly. Neocortical association areas accumulate no or few NFT, and primary motor, sensory and visual cortices are essentially free of NFT in nondemented elderly. Diffuse plaque accumulation in the neocortex in nondemented elderly is more variable and most frequently found in temporal, parietal and frontal cortices. Entorhinal cortex and hippocampus are not as frequently involved by SP. Neuritic plaques are found much less commonly in nondemented subjects and, when present, they are few in number. When they are present, they are most often found in conjunction with NFT accumulation. Thus, there is a spectrum of change in nondemented elderly from no to few neocortical diffuse SP, to others with abundant diffuse neocortical SP and hippocampal and entorhinal NFT and rare neocortical NFT and NP. Criteria for incipient AD are not available and whether the latter subjects have incipient AD must await further studies.

HETEROGENEITY OF ALZHEIMER'S DISEASE

Any consideration of criteria for AD must take into account the marked heterogeneity of histopathological findings observed in the disease. There is considerable variation in the density and location of NFT and SP ranging from a moderate number of patients having a predominance of SP to a small number of patients having a predominance of NFT with the majority having a mixture of SP and NFT. In two studies, 21 to 30% of AD cases had an abundance of SP and few or no neocortical NFT, termed SP predominant AD (40,54). Others have reported a subgroup of AD patients without neocortical NFT and abundant SP (32). Subsequently, another study showed that many of the patients with SP predominant form of AD have abundant neocortical Lewy bodies (LB) (24). Recently Ulrich et al. (56) described a group of older demented subjects whose brains contained abundant NFT and/or neuropil threads in the entorhinal region, hippocampus, and amygdala, but no neocortical NFT and few or no amyloid deposits. This has been referred to as senile dementia with tangles only, NFT predominant AD, or atypical AD. Subsequently, Bancher and Jellinger (3), in a series of 265 AD subjects, described 10 patients with abundant NFT in the limbic area but no or few neocortical NFT or SP. All of these patients were over 80-years old; four were severely demented, five were moderately demented, and one was mildly demented. We have observed at least four similar subjects in our autopsy material and several single cases have been observed by others (22,26). Whether this represents a subtype of AD, a stage in the development of typical AD, or a separate entity, requires further clinical-pathological studies.

In addition to the heterogeneity of relatively pure AD cases,

there is an overlap with other disorders such as diffuse Lewy body disease (DLBD), vascular dementia, dementia with argyrophilic grains, cranial trauma, and hippocampal sclerosis.

Diffuse Lewy body disease, Lewy body dementia, Lewy body variant of AD, or senile dementia of the Lewy body type are terms frequently used to describe the same entity. Diffuse Lewy body disease is a complicated neurological concept that is undergoing refinement. There are two differing views about this entity: a) that it is a variant of AD, and b) it is a distinct entity. Autopsy studies have revealed brainstem and neocortical LB in a significant percentage of AD cases (19,25,36). In the CERAD study, Parkinson's disease changes were present in 21% of AD cases (20). Lennox reviewed a series of neuropathological studies of dementia patients and found that 19% fulfilled the diagnosis of DLBD (35). Pure DLBD has abundant cortical and brainstem LB and no or insufficient NFT and SP to meet criteria for AD (37). However, many patients with abundant brainstem and neocortical LB also meet neuropathological criteria for AD. Lennox et al. (34) quantified LB in six cerebral regions and found a strong correlation between LB density and dementia severity, but they found no correlation with NFT density. Samuel et al. (51) quantified LB in patients with the "Lewy body variant of AD" compared with a group of classic AD patients without LB. They found lower neocortical NFT and NP counts in those with mixed AD pathology and LB than in classic AD cases without LB. Neocortical LB concentrations correlated significantly with dementia severity in the mixed pathology group. Recently, consensus guidelines for the classic and pathological diagnosis of dementia with Lewy bodies have been established by the Consortium on Dementia with Lewy bodies (41). Lewy bodies are most prominent in the cingulate gyrus, insula, amygdala, and medial temporal neocortex. They are best demonstrated by ubiquitin immunohistochemistry. The clinical picture of DLBD often includes intellectual decline of a fluctuating nature, psychotic symptoms (hallucinations, delusions, paranoia, and aggressive behavior), Parkinsonian features (bradykinesia, tremor, rigidity and shuffling gait), and occasionally, sensitivity to neuroleptic medications. The duration of survival is approximately six years. These cases probably represent part of a spectrum of neurodegenerative diseases with Parkinson's disease on one end, AD on the other, and DLBD in the middle (35).

Diagnostic neuropathological criteria do not exist for vascular dementia (VaD) or mixed VaD and AD. Although older reports suggest that pure VaD is the second most common cause of dementia, there is a paucity of clinical-pathological correlative studies to validate this. In several large autopsy studies of dementia patients, only a small number of cases ($< 2\%$) have been pathologically confirmed VaD (29,43). In our unreported autopsy series of 557 demented subjects, only 2.4% had VaD. It is my personal view that many of the patients diagnosed clinically with VaD actually have mixed VaD and AD and that pure VaD is rare. Twenty-eight percent of 92 cases in the CERAD study had infarcts or hemorrhage (20). In our study of 102 autopsies from the Nun Study, we found that of the 61 participants who met the neuropathologic criteria for AD, those with brain infarcts ($n = 24$) had poorer cognitive function than those without infarcts ($n = 37$) (53). Subjects with lacunar infarcts in the basal ganglia, thalamus, or deep white matter had an especially high prevalence of dementia compared with those without infarcts. Fewer AD lesions appeared to result in dementia in those with lacunar infarcts than those without infarcts. How many of these subjects would be classified as mixed AD and VaD must await the development of criteria for this entity. In the 41 participants who did not meet criteria for the neuropathologic diagnosis of AD, brain infarcts were only weakly associated with poor cognitive function. These findings suggest that brain infarcts as a contributing factor may play an important role in determining the severity of clinical symptoms in some patients with AD.

Argyrophilic grains are small spindle-shaped, silver-positive grains scattered throughout the neuropil in CA1, transentorhinal and entorhinal cortex, amygdala, and lateral tuberal nuclei. Dementia with argyrophilic grains is an adult onset type of dementia recently described by Braak and Braak (8,9). Subsequently, a few other cases of dementia with argyrophilic grains have been reported (28,39,57). The clinical symptoms of some patients with argyrophilic grains dementia are similar to AD, except that the course is somewhat shorter. Some individuals present with personality changes rather than memory loss and thus resemble Pick's disease. A few cases have shown argyrophilic grains and loss of neurons in the entorhinal pre-alpha region without AD neuropathology. Other patients have had argyrophilic grains with neurofibrillary change typical of AD. Thus, argyrophilic grains, when coexisting with AD, can be a confounding factor that should be recognized.

Hippocampal sclerosis is characterized by severe neuron loss and fibrous gliosis in CA1 and adjacent subiculum. Hippocampal sclerosis is found occasionally in patients with long-standing epilepsy or as a result of hypoxic/ischemic injury. Experimental animal studies and numerous human studies have established that the CA1 region is selectively vulnerable to hypoxic/ischemic injury. A recent report by Dickson et al., (17) of 81 autopsied brains from subjects 80 years of age or older revealed that 26% of demented patients exhibited hippocampal sclerosis. Of these, three subjects had AD, one had DLBD, four were associated with other vascular lesions, and four had no other obvious cause for dementia. They concluded that hippocampal sclerosis is a common post mortem finding in demented subjects but not in normal elderly subjects. The additive effect of hippocampal sclerosis in the dementia of AD is not understood and requires further evaluation. Because hippocampal sclerosis is found in demented individuals without other morphological causes for dementia, it suggests that it could be a causative factor on its own. It is clear that it could be a confounding factor in AD.

Traumatic brain injury also may be a confounding factor in AD. Cerebral trauma is recognized as a risk factor for AD (27,46). Cerebral trauma in the form of contusions, hemorrhages, lacerations, diffuse axonal injury, and hypoxic-ischemic lesions can lead to dementia (posttraumatic dementia), which generally is fixed or nonprogressive (13). In AD patients, it is quite likely that superimposed cerebral trauma, even of a mild nature, could cause worsening of clinical symptoms. Thus, at autopsy, all traumatic lesions should be recorded and an effort should be made to determine their relative correlation with clinical symptoms and progression.

RECOMMENDATIONS FOR CRITERIA FOR THE NEUROPATHOLOGICAL DIAGNOSIS OF ALZHEIMER'S DISEASE

Defining the criteria for the histopathologic diagnosis of AD has proven difficult because of the divergent opinions about this subject. Recent work has shown clearly that the criteria described by Khachaturian (33) should be replaced. Some aspects of the CERAD criteria should be changed. A simple approach would be to further refine the CERAD criteria and add: a) semiquantitation of NFT in neocortex, hippocampus, amygdala, and entorhinal cortex, and b) semiquantitation of NP in the neocortex, hippocampus, amygdala, and entorhinal cortex.

It is difficult to discriminate between preclinical, early or mild AD, and pathological aging changes in individuals with normal cognition. To date, too few cases of clinically well documented early AD have been autopsied to clearly define neuropathological

criteria for this group, and it is clear that these criteria must await further clinical-pathological correlative studies. Anecdotally, an autopsy of a 57-year-old female with a strong family history of dementia who had been functioning as a guidance counselor 3 months before her death from malignant melanoma revealed abundant neocortical and hippocampal NFT, NP, and diffuse plaques that exceeded criteria for the diagnosis of AD. A retrospective interview with her husband revealed minimal cognitive dysfunction, suggesting that early or preclinical cases of early-onset AD may have advanced histopathological changes.

Because AD is truly a clinical-pathological diagnosis, I favor making a diagnosis of AD only in the presence of a clinical history of dementia. Without the clinical history of intellectual decline, the diagnosis of AD is never absolutely certain, and we are left with vague modifying terms. Although this may be impractical, the problem caused by the absence of a clinical history of dementia has been a hindrance to establishing crisp criteria for AD in the past. Thus, I favor concentrating on definite AD and abandoning the CERAD criteria for "uncertain evidence of AD, suggestive of the diagnosis of AD, and possible or probable AD."

In an attempt to develop new criteria for the neuropathologic diagnosis of AD, it realistically should be remembered that the criteria will only define approximately 95% of patients correctly. There will always be a small number of demented subjects in whom a definitive diagnosis is not possible. In addition, there are subsets of AD that will not fit well into presently conceived criteria. Hopefully, these subsets and their relationship to AD will be defined more clearly in the future.

GROSS PATHOLOGY

The brain should be examined carefully and brain weight and gross abnormalities recorded. The degree of generalized or regional cerebral cortical atrophy and hippocampal atrophy should be rated semiquantitatively (as in the CERAD protocol: none, mild, moderate, or severe). The degree of atherosclerotic change in the major arteries at the base of the brain should be recorded along with the presence of any vascular abnormalities such as aneurysms or vascular malformations. The degree of ventricular enlargement and the intercaudate distance should be recorded. All pale or hemorrhagic infarcts, lacunae, and hemorrhages should be measured and recorded. Any abnormalities of the mammillary bodies or pallor of the substantia nigra and locus ceruleus should be recorded.

Initially, gross and histopathological assessment should be made to define the presence of other dementing illnesses including VaD, Pick's disease, Parkinson's disease, Creutzfeldt-Jakob disease, pure DLBD, progressive supranuclear palsy, corticobasal degeneration, progressive subcortical gliosis, frontal lobe degeneration of non-Alzheimer type, chromosome 17-linked dementia, Wernicke–Korsakoff disease, chronic meningoencephalitis, primary or secondary brain tumors, chronic subdural hematomas, and AIDS dementia.

HISTOPATHOLOGIC CRITERIA FOR THE DIAGNOSIS OF ALZHEIMER'S DISEASE

The modified Bielschowsky method, the Gallyas method, or the thioflavin S preparation should be used for SP and NFT detection. Immunocytochemical procedures using Aβ antibodies for amyloid, antiubiquitin antibodies for LB, tau antibodies for neuropil threads, and PHF-1 antibodies should be used as indicated. The Gallyas method works well for detecting argyrophilic grains and neuropil threads.

Neuritic plaque quantitation should be performed in 6–8 microns-thick silver or thioflavin S stained sections of the middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobule, hippocampus (CA1/subiculum) at the level of the lateral geniculate nucleus, entorhinal cortex (at mid-uncus), and amygdala using a × 10 objective. Plaque counts should be obtained from the five areas of maximum density in each region and a mean obtained for each (it might not be possible to obtain five different fields at × 10 in the CA1/subiculum). Quantitation in the amygdala should be performed in the accessory basal and central nuclei. The same sections should be used for NFT quantitation using a × 20 objective from the five areas of maximum density of NFT in each region and a mean obtained.

The criteria for the clinical diagnosis of AD should be those described by McKhann et al. (42). For a diagnosis of late stage definite AD, the patient should have a history of progressive dementia, a Mini-Mental State Examination (MMSE) score of less than 11, a Clinical Dementia Rating Scale score of 3 or more, or other neuropsychological testing showing late stage intellectual decline. The minimal histopathological findings should be as follows:

1. A mean of at least five NFT per × 200 (20 × objective) field in two of the three neocortical association areas (frontal, temporal, and parietal).
2. A mean of at least 10 NFT per × 200 (20 × objective) field in hippocampus (CA1/subiculum) and amygdala and a large number of NFT in the entorhinal cortex.
3. A mean of at least 15 neuritic plaques per × 100 (10 × objective) field in 2 of the 3 neocortical association areas (frontal, temporal, and parietal).

For a diagnosis of mid-stage definite AD, the patient should have a history of progressive dementia, a MMSE score of 11 to 17, a Clinical Dementia Rating Scale score of 1 to 2, or other neuropsychological testing indicating mid-stage intellectual decline. It seems reasonable to scale down the above histopathological findings slightly for a diagnosis of mid-stage definite AD, but the precise number of NFT and NP must await clinical-pathological correlative studies as described below.

CONFOUNDING FACTORS

In patients meeting the criteria for definite AD, other confounding findings such as the presence of LB, vascular lesions, hippocampal sclerosis, argyrophilic grains, or traumatic lesions should be identified. These could serve as additive factors to increase the cognitive decline, cause additional neurologic deficits, or lead to a diagnosis of a subset of AD. Each case should list the other pathological findings. If a patient meets criteria for definite AD, but has abundant LB in the substantia nigra, locus ceruleus, and neocortex, a diagnosis of AD plus LB or Lewy body variant form of AD should be made. If a subject has multiple vascular lesions in brain regions that are important in cognitive function or lesions totaling 50 ml or greater of brain infarction, a diagnosis of mixed AD and VaD should be made (the criteria for mixed AD and VaD need to be addressed). If hippocampal sclerosis is present, a diagnosis of AD with hippocampal sclerosis should be made. If argyrophilic grains are present, the resulting diagnosis should be AD with argyrophilic grains and, if cerebral contusions are present, they should be a part of the diagnosis. All major overlapping or confounding disorders should be treated in the same way so that subsets of AD or confounding factors can be identified.

It is tempting to propose two sets of criteria: one for research investigators and a second less rigorous one for practicing pathologists. However, this is quite difficult and it would be more desirable to revise the present criteria, validate them, and then

establish semiquantitative scales for practicing pathologists. It is proposed that a series of 150 new, prospectively evaluated, dementia cases of varying severity with the clinical diagnosis of AD be evaluated by participants in this project to assess these criteria. This could be organized in the Alzheimer's Disease Centers or as part of CERAD. All confounding disorders, such as noted above, should be excluded in this group of cases so that pure definite AD cases will be used to validate the criteria recommended by participants in this workshop.

The above part of this position paper was written prior to the Workshop on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. The Workshop proved to be a successful discussion of issues, and a consensus was reached. The major changes in the criteria are: a) elimination of the previous NIA-NINCDS (Khachaturian) criteria, b) use of semiquantitation of NP and elimination of diffuse plaques for diagnosis, c) addition of semiquantitation of neocortical NFT, d) addition of the examination of the hippocampus and entorhinal cortex, e) emphasis on defining coexisting pathological lesions, f) establishment of criteria for general pathologists and more rigorous criteria for AD research setting, and g) use of the Braak and Braak topographic staging method (10) for research centers. Overall, I believe that these represent significant improvements in the criteria. The workshop also recommended important areas for future goals to improve the postmortem diagnosis of AD. There are weaknesses in the criteria that will have to be addressed in the future. They clearly will not recognize plaque-predominant AD or the rare NFT-predominant AD. Criteria for incipient or early AD are not recommended but remain an important area for future study. The presence or absence of a clinical history of dementia and the ages of the patients are not clearly addressed. Although not without flaws, the new criteria will serve as guidelines for study and future refinement until a molecular or biochemical diagnostic test is established for the diagnosis of AD.

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