Neuropathologic Substrate of Mild Cognitive Impairment

William R. Markesbery, MD; Frederick A. Schmitt, PhD; Richard J. Kryscio, PhD; Daron G. Davis, MD; Charles D. Smith, MD; David R. Wekstein, PhD

Objective: To define the neuropathologic findings in amnestic mild cognitive impairment (MCI) and early Alzheimer disease (EAD).

Methods: The mean numbers of diffuse plaques, neuritic plaques (NPs), and neurofibrillary tangles (NFTs) in 4 neocortical regions and 4 ventromedial temporal lobe regions were counted in 10 patients with amnestic MCI and compared with the mean numbers in 23 normal control subjects and 10 patients with EAD, and then were compared with memory performance. All of the controls and patients were followed longitudinally.

Results: Patients with MCI showed no significant difference (P>.05) in the number of diffuse plaques from that in normal controls or patients with EAD. In patients with MCI, the number of NPs was significantly elevated in all 4 neocortical regions and amygdala compared with controls (P<.01 to <.001). There were no significant differences (P>.05) in the number of NPs between MCI and EAD cerebral cortex, but significant increases were present for NPs in EAD amygdala and subiculum compared with MCI (P<.01). In patients with MCI compared with controls, the only significant increase in NFTs in the neocortex was in the parietal lobe. However, the number of NFTs was significantly elevated in MCI in all 4 ventromedial temporal lobe structures compared with controls (P<.01 to <.001). In comparing MCI with EAD, there were significant increases in NFTs in EAD in frontal and temporal lobes, amygdala, and subiculum (P<.01). The numbers of NPs and NFTs were significantly elevated in all of the neocortical regions and ventromedial temporal lobe regions in patients with EAD compared with controls (P<.001). Memory function was significantly correlated with NFTs in CA1 of the hippocampus (P<.01) and the entorhinal cortex (P<.05).

Conclusions: In patients with amnestic MCI who were followed longitudinally, the early changes of Alzheimer disease were present. The NFTs were slightly more prominent than β-amyloid peptide deposition in the progression from normal to MCI to EAD. Ventromedial temporal lobe NFTs probably represent the substrate for memory decline in MCI. From a neuropathologic perspective, it appears that amnestic MCI is, in reality, EAD.

Arch Neurol. 2006;63:38-46

THE RECENT EMPHASIS IN ALZHEIMER disease (AD) clinical research is on early disease detection, with the hope of early treatment to slow progression. Slowing the onset or progression of AD could prevent many individuals from developing symptoms and save billions of dollars in health care costs. The concept of mild cognitive impairment (MCI) as a phase between normal aging, early dementia, and AD has served as an added stimulus for early detection. The term MCI was initially used by Flicker et al,1 who showed progression to dementia in a group of elderly subjects who were longitudinally followed and initially had mild impairment on psychometric tests when compared with controls. Subsequently, Petersen and colleagues2-3 refined the concept in more detail. Amnestic MCI is characterized by memory complaints, objective evidence of memory impairment for age and education, intact general cognitive function, minimal changes in activities of daily living, and lack of dementia. Other forms of MCI exist, including multiple-domain and single-memory-domain types, but amnestic MCI appears to be the most common form.4 Descriptions of the rate of the progression of MCI and the stability of MCI as a syndrome vary considerably across different studies, in part because of differing subject populations.5-10 There have been few autopsies of individuals with MCI. Further, in those individuals who die before dementia is clinically diagnosed, there is often a long lag between the last clinical evaluation and...
death, making diagnosis less certain. Only a few neuropathologic studies have longitudinally followed are available, and they comprise only a small number of cases. These studies have shown a wide spectrum of neuropathologic changes in this entity, and the neuropathologic substrate of MCI has not been determined. One of the difficulties in determining the neuropathologic changes in MCI is that different groups apply various names and diagnostic criteria to this clinical entity, including aging-associated cognitive decline, cognitive impairment, no dementia, mild AD, very early AD (EAD), and preclinical AD, and each study described different findings. This article defines the neuropathologic changes in the brain in 10 patients with amnestic MCI who met the criteria described by Petersen et al, 10 patients with EAD, and 23 normal control subjects. All of the patients were followed longitudinally in our Alzheimer’s Disease Center’s research clinic, University of Kentucky, Lexington, and in our normal control clinic.

METHODS

SUBJECTS

The patients with MCI and normal control subjects were from our normal control group in which we performed 163 autopsies. All of the control subjects as well as patients with MCI and EAD were from autopsies of 70 control subjects and 149 patients with dementia that were obtained from January 1, 2000, to July 1, 2004. The details of the recruitment, inclusion criteria, and mental status test battery for our normal control group have been described previously. All of the subjects were contacted at 6-month intervals, had detailed mental status testing annually, and had neurologic and physical examinations biannually. Subjects had been followed for 1 to 14 years (median, 10.9 years). Once the subjects transitioned to having MCI or EAD, they received the mental status test battery and neurologic evaluation annually.

The 23 normal subjects were cognitively intact on enrollment and remained so during follow-up. Numerous normal subjects in our control group have had abundant AD neuropathologic changes with Braak scores of III to VI, and some investigators have referred to subjects such as these as having preclinical AD. Thus, for this study, we chose only those with Braak scores of II or below to represent the most normal control subjects. We did not think that it was appropriate to compare patients with MCI with subjects with preclinical AD whose pathologic findings might make them a step closer to MCI. The 10 subjects with MCI were initially normal on enrollment into our longitudinal study and later developed MCI during follow-up. All except 1 of our patients with EAD were from our control clinic, were initially normal, and gradually progressed to having EAD. One patient with EAD was from our Alzheimer’s Disease Center’s research clinic and was not cognitively normal at the initial visit. This subject had EAD at the last evaluation before death.

The demographic data and other information about control subjects and patients with MCI and EAD are presented in the Table. The control group comprised 9 women (mean ± SD age, 83.0 ± 7.1 years) and 14 men (mean ± SD age, 81.3 ± 7.5 years). The MCI group comprised 7 women (mean ± SD age, 90.1 ± 4.7 years) and 3 men (mean ± SD age, 87.7 ± 1.2 years). The EAD group comprised 6 women (mean ± SD age, 87.3 ± 6.7 years) and 4 men (mean ± SD age, 84.0 ± 2.4 years). The mean ± SD postmortem interval of 6.3 ± 5.8 hours for patients with EAD was not significantly longer than for controls (3.9 ± 2.8 hours) or patients with MCI (4.2 ± 2.8 hours), although 2 patients with EAD had postmortem intervals of 14.5 hours and 17.2 hours. The length of time from diagnosis to death was 2.6 years (median, 2.0 years) for patients with MCI and 5.1 years (median, 5.0 years) for patients with EAD. With the exception of 1 African American patient in the EAD group, all of the other subjects in this study were white. The most frequent causes of death were myocardial infarction, pneumonia, pulmonary emboli, cancer (none had cerebral metastases), chronic obstructive pulmonary disease, and congestive heart failure. Five control subjects, 3 patients with MCI, and 3 patients with EAD were heterozygous for the apolipoprotein E4 allele, and 1 patient with EAD was homozygous.

COGNITIVE EVALUATIONS

The mental status testing of our subjects has been described previously. The tests of cognition included the mental status procedures of the Consortium to Establish a Registry for Alzheimer’s Disease, some of the procedures used in the Washington University Memory and Aging Project testing battery, and those used by Eslinger et al. These were supplemented by the vocabulary and digit symbol subtests from the Wechsler Adult Intelligence Scale–Revised at baseline and the Trail-Making Test from the Halstead-Reitan Neuropsychological Battery.

DIAGNOSES

Control subjects had no cognitive complaints, normal cognitive test scores (especially objective memory test scores), intact activities of daily living, and normal neurologic examinations. All of the control subjects were Braak stage II or lower. The criteria used for MCI were described by Petersen et al. These criteria include a memory complaint corroborated by an informant, objective memory test impairment (age and education adjusted), general normal global intellectual function, gen-
Neurofibrillary tangles (NFTs), diffuse plaques (DPs), and neuritic plaques (NPs) were quantified in the amygdala, CA1, subiculum, and entorhinal cortex. Comparison of the modified Bielschowsky–stained sections and the Gallyas-stained sections in ventromedial temporal lobe structures revealed that the Gallyas stain detected more NFTs, but this reached statistical significance only in the entorhinal cortex \( (P < .05) \). Thus, we used the Gallyas stain to quantify NFTs in medial temporal lobe structures. Senile plaques were counted using a 10× objective (field size, 2.35 mm\(^2\)) in the 5 most involved fields in each section of the regions described earlier. The most involved fields were determined by studying the whole section and marking it. Fewer fields were counted in hippocampal CA1 and subiculum because of the small size of these structures. Senile plaques were separated into DPs (plaques without neurites) and NPs (plaques with neurites) in each region. Neurofibrillary tangles were counted with a 20× objective (field size, 0.586 mm\(^2\)) in the 5 most involved fields of each section of the regions described earlier. An arithmetic mean was calculated from the count of the 5 most involved fields for DPs (number of DPs per 2.35 mm\(^2\)), NPs (number of NPs per 2.35 mm\(^2\)), and NFTs (number of NFTs per 0.586 mm\(^2\)) for each region.

### Statistical Analysis

Comparison of the control, MCI, and EAD groups for DPs, NPs, and NFTs was performed using Kruskal–Wallis nonparametric analyses of variance. Statistical significance was set at \( P < .01 \) for a conservative evaluation of differences of all the main and post hoc analyses.

### Results

Scatter plots are not shown for DPs to keep the number of plots manageable and because few significant results were present for DPs.

### Comparison of Controls and MCI

There were no significant differences in the number of DPs in controls and in patients with MCI in the neocortex or medial temporal lobe structures. Neuritic plaque counts were significantly elevated in MFG \( (P < .001) \), MTG \( (P < .01) \), IPL \( (P < .01) \), PCG \( (P < .01) \) (Figure 1), and amygdala \( (P < .01) \) (Figure 2) in MCI compared with normal controls. No differences were found in the number of NPs between the MCI and control groups in the hippocampal CA1, subiculum, or entorhinal cortex \( P > .10 \) to \( > .05 \).

In the neocortex, NFTs were significantly increased in only the IPL \( (P < .01) \) in patients with MCI compared with controls (Figure 3). The number of NFTs was significantly elevated in patients with MCI compared with controls in the amygdala \( (P < .01) \), entorhinal cortex \( (P < .001) \), CA1 \( (P < .001) \), and subiculum \( (P < .001) \) (Figure 4).

### Comparison of MCI and EAD

No significant differences were found in the number of DPs between the MCI and EAD groups except for an increase in MTG in patients with EAD \( (P < .01) \). There was no significant difference in the number of NPs in MCI and EAD cerebral cortex \( (P > .05) \) (Figure 1). Significant elevations were present in the number of NPs in EAD amygdala \( (P < .01) \) and subiculum \( (P < .01) \) compared with MCI (Figure 2). In EAD, significant increases in NFTs...
were present in the MFG (P<.01) and MTG (P<.01) (Figure 3) as well as in the amygdala (P<.01) and subiculum (P<.01) (Figure 4) compared with MCI.

COMPARISON OF CONTROLS AND EAD

There were significant increases in DPs in all of the neocortical and ventromedial temporal regions except the IPL in EAD compared with controls. Neuritic plaques were significantly elevated in all of the neocortical areas and ventromedial temporal lobe structures (all P<.001) in EAD compared with controls (Figure 1 and Figure 2). The number of NFTs was significantly elevated in all of the neocortical regions and ventromedial temporal lobe structures in EAD compared with controls (P<.001) (Figure 3 and Figure 4).

OTHER PATHOLOGIC LESIONS

Twelve control subjects did not have any cerebrovascular lesions (infarcts or hemorrhages). Six control subjects had 1 or more microinfarcts, which were mostly chronic. Three subjects had small- to moderate-sized cystic cerebral infarcts (the largest was 6 cm). One subject had a lacunar infarct. One control subject had an aneurysm of the middle cerebral artery (clipped in the distant past) and had small circumjacent areas of encephalomalacia in the frontal and temporal lobes.

Three patients with MCI had no cerebrovascular lesions, 1 had a lacunar infarct in the caudate nucleus, 4 had 1 or more microinfarcts of variable age (many were acute), and 2 had acute microhemorrhages or tiny hemorrhages. One patient with EAD had no cerebrovascular lesions. Nine patients with EAD had microhemorrhages, microinfarcts, lacunar infarcts, or small pale or hemorrhagic infarcts. Many of these were tiny, and some were acute. None of our subjects had hippocampal sclerosis.

Four control subjects had small to moderate numbers of AGs in ventromedial temporal lobe structures. Three patients with MCI had small to moderate numbers of AGs in ventromedial temporal lobe structures, including the ambient gyrus. All of the patients met only stage 1 or stage 2 classifications of Saito et al when Gallyas staining of the gyrus rectus, insula, and anterior cingulate gyrus was added. Two patients with EAD had AGs in small numbers in ventromedial temporal lobe structures. The α-synuclein staining revealed that 2 control brains contained Lewy bodies. One met the criteria for the limbic form of dementia with Lewy bodies using the consensus guidelines for the pathologic diagnosis of dementia with Lewy bodies. The brain of 1 patient with EAD contained Lewy bodies in a sufficient number to also meet the criteria for the limbic form of dementia with Lewy bodies. Two patients with EAD had rare Lewy bodies; one patient had a single Lewy

Figure 1. Scatter plots of neuritic plaque counts in neocortical regions of normal control subjects (NRM), patients with mild cognitive impairment (MCI), and patients with early Alzheimer disease (EAD) in the frontal lobe (A), temporal lobe (B), parietal lobe (C), and posterior cingulate gyrus (D). Each square and triangle represents the mean of the 5 most involved fields in each region (field size, 2.35 mm²). The straight horizontal line indicates the mean; asterisk, the number of neuritic plaques is significantly greater in EAD than in NRM; and dagger, the number of neuritic plaques is significantly greater in MCI than in NRM. Statistical P values are given in the “Results” section.
body in the amygdala, and the other had 4 in the temporal pole section.

CORRELATION WITH MEMORY TESTING

Associations between average counts for DPs, NPs, and NFTs with delayed verbal recall using the Consortium to Establish a Registry for Alzheimer’s Disease 10-item word list by all of the cases were explored using Pearson correlation coefficients adjusted for multiple comparisons (Bonferroni). Correlations between delayed memory performance and NFTs ranged from −0.50 for NFTs in the CA1 region (adjusted \( P < .01 \)) and −0.44 in the entorhinal cortex (adjusted \( P < .05 \)) to nonsignificant correlation coefficients in the remaining regions (\( P > .05 \)). For DPs and NPs, correlation coefficients did not reach statistical significance (\( P > .05 \)).

COMMENT

This study only used normal control subjects and patients with MCI and EAD who were thoroughly evaluated and followed longitudinally. It should be underscored that our subjects were quite elderly, well educated, and had volunteered for our study. All of the patients with MCI and all but 1 patient with EAD had been followed from normal cognitive function through their transition to amnestic MCI, EAD, or both. The length of time between their last mental status testing and death was no longer than a mean of 9.3 months for any of the groups. All of the final clinical diagnoses were made through clinical-pathologic correlate conferences that used clinical, neuropsychological, radiological, and laboratory data to determine the clinical diagnoses prior to any discussion of neuropathologic data. There are no criteria available for the neuropathologic diagnosis of MCI or EAD. Neuropathologic data were used only to support or clarify the presence of a disease state after the clinical diagnosis was determined.

This study showed no significant differences in the number of DPs between the MCI and control groups, as well as no significant differences in the number of DPs between the MCI and EAD groups except in the MTG, where there was a significant increase with EAD. Because DPs were so common in normal control subjects, our data suggest that DPs are not critical neuropathologic determinants of the transition from normal to MCI or MCI to EAD.

One of the major differences in the transition from controls to having MCI was a significant increase in NPs in all of the neocortical regions and amygdala with MCI. However, there were no differences in the number of NPs...
between the MCI and EAD groups except for a significant increase in EAD amygdala and subiculum. This indicates that the pathologic deposition of insoluble β-amyloid peptide and formation of neurites in cerebral cortex progress from normal to MCI, but in contrast, they do not distinguish MCI from EAD.

Except for an increase in the number of NFTs in the IPL in patients with MCI, there was no significant difference in neocortical NFT formation between patients with MCI and control subjects. Another major difference in the transition from being normal to having MCI was the significant increase in the number of NFTs in the entorhinal cortex, CA1, subiculum, and amygdala. Neuropathologic progression is implied by the significant increase in NFTs in the MFG, MTG, amygdala, and subiculum in EAD compared with MCI. The increase in NFTs in all of the neocortical and ventromedial temporal lobe structures in EAD compared with controls further supports a gradual increase in NFT formation from normal aging to having MCI to having EAD.

The major neuropathologic changes in MCI are the numbers of NPs in the neocortex and NFTs in ventromedial temporal lobe structures. Neurofibrillary tangles increase in the transition from MCI to EAD, and they begin to incorporate neocortical regions. This is in keeping with the changes described by Braak and Braak, who initially demonstrated that the pathologic process in AD begins in the entorhinal cortex, progresses to the hippocampus, and then progresses to other brain regions in a topographically predictable manner. In the transentorhinal stage (I to II), NFTs are initially in the entorhinal region and subsequently in lamina II (pre-α) of the entorhinal cortex and in hippocampal CA1. These changes alone usually do not have a clinical signature. All of our control subjects were Braak stage I to II with a mean Braak score of 1.4, and none showed clinical evidence of cognitive decline. In the limbic stage (III to IV), there is an increase in neurofibrillary pathology in the entorhinal cortex and CA1, as well as early involvement of the adjacent temporal neocortex and the amygdala. In stage IV, the neurofibrillary pathology becomes more prominent in the regions described earlier, and it spreads to the subiculum. Some patients with these changes may show cognitive decline, such as that found in our patients with MCI. The mean Braak score for our 10 patients with MCI was 3.3, with a range from stage II to V. The entorhinal cortex is a gateway to the hippocampus, shuttling information in and out of this region. The hippocampus takes information into short-term memory and relays it to brain areas for processing and long-term storage. Thus, neurofibrillary pathology in the entorhinal cortex and hippocampal CA1 coupled with the neuron loss in the entorhinal cortex described by others probably correlates with memory decline in amnes-
tic MCI. The significant correlation of memory decline with NFTs in the entorhinal cortex and CA1 in our study supports this concept.

Our patients with EAD were Braak stage V (isocortical stage) except for 1 case, although there was no significant increase in NFTs in the entorhinal cortex or CA1 in EAD compared with MCI. Braak and Braak have suggested that subicular NFTs develop later than CA1 NFTs, and our finding that subicular NFTs increase in the transition from MCI to EAD support that concept. Overall, the transition from MCI to EAD appears to be more related to NFT formation in the neocortex and ventromedial temporal lobe structures than to insoluble β-amyloid peptide deposition.

A few of the patients with MCI and EAD have other mild pathologic alterations, including cerebrovascular lesions, AGs, and Lewy bodies. Similar findings were present in controls. None of the patients with AGs met the criteria for AG dementia. The mean age for patients with AGs ranged from 86 to 88 years. Our previous studies and those of others have suggested that in most instances, AGs are an age-related phenomenon when not present in widespread regions in large numbers. Although there were other pathologic findings in our patients with MCI and EAD, they were not sufficient to lead to another diagnosis. These changes are also present in normal controls, and whether they influence cognition in an additive manner in patients with MCI and EAD remains to be determined.

There have been only a few neuropathologic studies of patients with MCI who have been studied longitudinally with frequent neuropsychologic assessment and physical and neurologic examinations. In a study of patients who were followed longitudinally and had CDR scores of 0.5, Price and Morris found that all of the patients had the neuropathologic changes of AD. They also found neocortical and limbic DPs, NPs, and NFTs in non-demented cases and suggested that the patients had “preclinical” AD. Morris et al found that a series of subjects with CDR scores of 0.5 with “incipient AD” at entry almost always had neuropathologic features of AD at autopsy (2 of the patients had vascular dementia, and 1 had frontotemporal dementia). In another brief description of 11 subjects with the clinical diagnosis of MCI, 5 had AD-like pathology with NFTs in the medial temporal lobe and a moderate number of DPs and sparse NPs in the neocortex. Two patients had NFTs involving medial temporal lobe structures only.

Several morphologic studies of patients with MCI who were followed longitudinally from the Religious Order Study have been published. Mufson et al found the β-amyloid peptide load in the entorhinal...
cortex in MCI to be intermediate between normal controls and patients with AD, but none differed statistically from the other, and they suggested that β-amyloid peptide deposition is not the major pathologic alteration underlying cognitive decline. Our study supports this conclusion. In another study, Kordower et al \(^{15}\) demonstrated a significant decline in the number and atrophy of entorhinal cortex lamina II neurons in patients with MCI compared with controls, but not when compared with patients with mild or moderate AD. In another article from the Religious Order Study, Mitchell et al \(^{12}\) described a significant increase in NFTs and neuropil thread density in the entorhinal cortex and perirhinal cortex in MCI and AD compared with controls.

Guillozet et al \(^{11}\) described the neuropathologic findings in 3 patients with MCI and 5 normal controls who had been followed longitudinally. In patients with MCI, NFTs were significantly more numerous in medial temporal lobe structures and showed a relationship to memory tests. Riley et al \(^{10}\) described the neuropathologic findings in a series of 130 elderly women in the Nun Study with a continuum of cognitive states, including 17 with MCI. This study suggested that NFTs are the major neuropathologic substrate of MCI. Thus, most autopsy studies of longitudinally evaluated patients with MCI have found that NFTs are the predominant microscopic lesions associated with this clinical classification that encompasses memory impairment and precedes the development of dementia. Our findings comparing patients with MCI with controls are in agreement with these studies, and they suggest a continuum of NFT pathology underlying transitions between normal aging, MCI, and EAD.

The changes in the PCG in our subjects are of interest in relation to the diminished cerebral blood flow described in this region in positron emission tomographic scans of patients with MCI. \(^{45}\) We found no significant increase in DPs or NFTs in the PCG in patients with MCI compared with control subjects or in patients with MCI compared with patients with EAD. Thus, diminished cerebral blood flow may not correlate with significant pathologic findings in the PCG. However, there were significant changes in ventromedial temporal lobe structures, especially in the entorhinal cortex and CA1 in MCI and EAD. The entorhinal cortex and CA1 are the sites of afferent projections from the PCG. \(^{40,41}\) It is possible that the early reduced cortical metabolism found in the PCG is related to disconnection of this region from the entorhinal cortex and CA1, as suggested by Huang et al. \(^{15}\)

In summary, our study indicates that in patients with amnestic MCI who are followed longitudinally, the early changes of AD are present. From a neuropathologic perspective, we are in agreement with Morris et al. \(^{16}\) who suggested that amnestic MCI is, in reality, early AD. This study underscores a need for a biomarker for the presence of AD pathology before the early symptomatic phase of amnestic MCI. If preventive measures are to be efficacious in AD, our study suggests that they should be initiated much earlier in the preclinical phase of the disease or early in the life of those at high risk for the disease.

Accepted for Publication: April 22, 2005.
Correspondence: William R. Markesbery, MD, 101 Sanders-Brown Center on Aging, University of Kentucky, 800 S Limestone St, Lexington, KY 40536-0230 (wmark0 @email.uky.edu).

Author Contributions: Study concept and design: Markesbery and Schmitt. Acquisition of data: Markesbery, Davis, Smith, and Wekstein. Analysis and interpretation of data: Markesbery, Schmitt, and Kryscio. Drafting of the manuscript: Markesbery. Critical revision of the manuscript for important intellectual content: Markesbery, Schmitt, Kryscio, Davis, Smith, and Wekstein. Statistical analysis: Schmitt and Kryscio. Obtained funding: Markesbery. Administrative, technical, and material support: Markesbery, Schmitt, and Wekstein. Study supervision: Markesbery and Schmitt.

Funding/Support: This study was supported by grant 5-P50-AG05144 from the National Institutes of Health, Bethesda, Md, and a grant from the Abercrombie Foundation.

Acknowledgment: We are deeply grateful to all of the participants in our longitudinal aging study and to the patients with Alzheimer disease in our Alzheimer’s Disease Center’s research clinic. We thank Ela Patel, Ann Tudor, Huaichen Liu, MD, Paula Thomason, Sonya Anderson, Sally Malley, Cecil Runyons, and Paula Neal-Thomas for technical support, and Gregory Cooper, MD, PhD, and Allison Caban-Holt, PhD, for clinical evaluations.

REFERENCES
