

Evaluation of heme oxygenase-1 as a systemic biological marker of sporadic AD

H.M. Schipper, MD, PhD, FRCPC; H. Chertkow, MD, FRCPC; K. Mehindate, PhD; D. Frankel, MSc; C. Melmed, MD; and H. Bergman, MD

Article abstract—*Background:* Heme oxygenase-1 (HO-1) is a 32-kDa stress protein that catalyzes the degradation of heme to biliverdin. HO-1 immunoreactivity is greatly increased in neurons and astrocytes of the hippocampus and cerebral cortex of individuals with AD and colocalizes to senile plaques and neurofibrillary tangles. *Methods:* We investigated whether systemic HO-1 regulation is also deranged in AD patients and whether blood HO-1 measurements provide a peripheral biomarker of the disease. Plasma HO-1 protein levels were measured by competitive ELISA and lymphocyte HO-1 mRNA levels were determined by Northern analysis in patients with early probable sporadic AD, normal elderly controls (NEC), normal younger controls, individuals with age-associated cognitive decline (AACD) not meeting AD criteria, and patients with non-Alzheimer dementia, nondementing neurologic illness, and chronic medical disorders. CSF HO-1 protein concentrations were also determined by ELISA in pathologically confirmed AD and control cases. *Results:* Mean plasma HO-1 protein concentrations were significantly lower in AD patients ($0.85 \pm 0.14 \mu\text{g/mL}$) compared with NEC ($1.77 \pm 0.34 \mu\text{g/mL}$; $p < 0.05$) and control patients. The AACD group exhibited plasma HO-1 concentrations ($1.06 \pm 0.33 \mu\text{g/mL}$) intermediate between, but not different from, those of the AD patients and NEC. Lymphocyte HO-1 mRNA levels were lower in the AD cohort relative to NEC ($p < 0.001$) and individuals with AACD, non-Alzheimer dementia, nondementing neurologic illness, and chronic medical conditions. Lymphocyte HO-1 mRNA levels were also lower in the AACD group relative to NEC ($p < 0.05$). In comparison with all groups excluding AACD, the sensitivity and specificity of lymphocyte HO-1 mRNA measurement for diagnosis of early sporadic AD are 88% and 75%. Mean CSF HO-1 protein concentrations were lower ($p < 0.01$) in AD cases (19.07 ng/mL) relative to control values (32.48 ng/mL). *Conclusions:* Plasma and CSF HO-1 protein and lymphocyte HO-1 mRNA levels are decreased in subjects with sporadic AD. Quantitative assay for lymphocyte HO-1 mRNA expression may serve as a useful biologic marker in early sporadic AD. **Key words:** Aging—AD—Biologic marker—Dementia—Diagnosis—Heme oxygenase-1.

NEUROLOGY 2000;54:1297–1304

Although definite AD can only be determined pathologically, clinically standardized criteria for the diagnosis of probable AD in life¹ correctly identify the disease in 80 to 90% of cases.²⁻⁴ The term mild cognitive impairment (MCI)⁵ is generally applied to elderly individuals who experience cognitive decline that fails to meet the clinical criteria of AD or other dementia. This level of mild impairment can be more rigorously defined as age-associated cognitive decline (AACD) when the patient exhibits deficits in only one of several cognitive domains (usually memory) of at least 6 months' duration that are demonstrable on testing to be at least one SD below values for age-matched normal controls. Although some AACD/MCI cases will progress to probable AD over the ensuing 3 to 5 years,^{6,7} many such individuals exhibit a sta-

ble, nonprogressive memory deficit over a long period of follow-up.⁸ The advent of a biologic marker that differentiates early, sporadic AD from normal aging and other dementing illnesses and identifies those MCI/AACD individuals who are destined to deteriorate to Alzheimer dementia would represent an achievement in the evaluation and management of this debilitating neurodegenerative disorder.⁹ Genetic markers, such as mutant forms of amyloid precursor protein, presenilin-1 and presenilin-2, are excellent for predicting disease in some kindreds with familial AD¹⁰ but have little or no role in the management of patients with the far more common sporadic form of the illness.¹¹ Several laboratories have demonstrated abnormally low levels of $A\beta_{1-42}$ ^{12,13} and increased concentrations of tau¹⁴ and neuropil thread

From the Bloomfield Centre for Research in Aging (Drs. Schipper, Chertkow, and Mehindate, and D. Frankel), Lady Davis Institute for Medical Research; the Departments of Clinical Neurosciences (Drs. Schipper, Chertkow, and Melmed) and Medicine, Division of Geriatrics (Drs. Schipper, Chertkow, and Bergman), Sir Mortimer B. Davis Jewish General Hospital, Montreal; the Department of Neurology and Neurosurgery (Drs. Schipper, Chertkow, and Melmed), McGill University, Montreal; and the Research Centre (Dr. Chertkow), Centre Hospitalier Côte-des-Neiges, Montreal, Canada. Supported by the Medical Research Council of Canada (H.M.S.), Alzheimer's Society of Canada (H.C., H.B.), Alzheimer's Association (US; H.M.S.), and the Fonds de la Recherche en Santé du Québec (H.M.S., H.C., H.B.).

Presented in part at the 27th annual meeting of the Society for Neuroscience; New Orleans, LA; October 23–30, 1997, and the 51st annual meeting of the American Academy of Neurology; Toronto; April 17–24, 1999.

Received February 26, 1999. Accepted in final form November 13, 1999.

Address correspondence and reprint requests to Dr. Hyman Schipper, Lady Davis Institute, Jewish General Hospital, 3755 Cote Ste. Catherine Road, Montreal, Quebec, Canada H3T 1E2.

protein¹⁵ in the CSF of sporadic AD patients. However, CSF examination by lumbar puncture is relatively invasive and therefore not suitable for mass screening of elderly individuals with AD risk factors or mild memory impairment. Increased plasma A β ₁₋₄₂ levels have been documented in several kindreds with familial AD¹⁶ but, as mentioned above, these families represent a very small subset of the total AD population. In 1996, Kennard et al.¹⁷ reported increased serum levels of the iron-binding protein p97 (melanotransferrin) in Canadian and Japanese patients with sporadic AD. However, the degree of overlap between AD and control cases was subsequently determined to be greater than initially surmised and further evaluation of p97 as a potential AD marker will be required.¹⁸

Heme oxygenase-1 (HO-1) is an enzyme that catalyzes the oxidative degradation of heme to biliverdin in brain and other tissues.¹⁹ This 32-kDa member of the heat shock protein superfamily is rapidly up-regulated by oxidative stress, metal ions, amino acid analogues, and sulfhydryl agents. In response to oxidative stress, induction of HO-1 may protect cells by catabolizing pro-oxidant metalloporphyrins, such as heme, to bile pigments (biliverdin, bilirubin) with free radical scavenging capabilities.²⁰ Conversely, free iron and carbon monoxide generated from HO-1-mediated heme catabolism may contribute to the abnormal patterns of brain iron deposition and mitochondrial insufficiency documented in various human neurodegenerative disorders.²¹ HO-1 immunoreactivity is markedly increased in neurons and astrocytes of post-mortem hippocampus and temporal cortex derived from AD subjects relative to normal control specimens matched for age and postmortem interval.²² Furthermore, we²² and others²³ have demonstrated colocalization of HO-1 protein to neurofibrillary tangles and senile plaques in AD specimens. In the current study, we set out to determine whether HO-1 mRNA and protein are also abnormally expressed in the blood and CSF of AD patients.

Methods. *Subjects.* This study was approved by the Research and Ethics Committee of the Sir Mortimer B. Davis Jewish General Hospital (JGH). Written informed consent was obtained from all patients (n = 175) or their primary caregivers. Fifty patients meeting the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable AD were recruited from the JGH/McGill University Memory Clinic, a tertiary care facility for the evaluation of memory loss in Montreal. Dementia in the AD cohort was mild to moderate in severity, with a mean Folstein Mini-Mental State Examination (MMSE) score of 21.1 of 30.²⁴ Twenty-five individuals meeting World Health Organization clinical criteria for AACD⁵ were recruited from the same clinic. All AACD subjects exhibited a score of 0.5 on the Clinical Dementia Rating (CDR) scale,²⁵ indicating questionable dementia. No individuals with AD or AACD were taking vitamin E, Ginkgo biloba extract, or other antioxidant supplements at the time of the study. Twenty-four normal elderly controls (NEC) were recruited from family practice clinics at the

same hospital. None had memory complaints and all scored within one SD of age- and education-standardized normal values on a series of memory and attention tests (CDR score 0.0).²⁵ Seven normal younger controls aged 40 to 60 years were recruited from sectors of the Montreal community typically serviced by the JGH. Sixteen patients with non-Alzheimer dementia were recruited from the McGill Memory Clinic, JGH wards, and Maimonides Hospital Geriatric Center (Montreal). The mean MMSE score for the non-Alzheimer dementia group as a whole was 11.5 of 30. Twenty-one nondemented patients with idiopathic PD were recruited from the JGH Movement Disorder Clinic. Fourteen patients with ALS were enrolled with the assistance of the ALS Association of Quebec. Of these, 13 had sporadic and 1 had familial ALS. Five ambulatory, nondemented individuals who sustained cerebral infarctions within the preceding 24 months were recruited from a general neurology clinic at JGH. Eight patients with chronic rheumatologic disorders and five with chronic liver disorders were recruited from JGH internal medicine clinics (table).

Neuropsychological testing. The following tests were performed in all cases of AD and AACD: Geriatric Depression Scale-Yesavage, Orientation score, Constructional Praxis score, A & J Word/Picture Matching test, Gestalt Closure subtest, Trail Making A, Clock Drawing, Wechsler Adult Intelligence Scale-Revised, Boston Naming Test, Benton Word Fluency test, Wechsler Memory Scale-Revised, and the Rey Auditory Verbal Learning test.

Blood samples. Whole blood was collected by phlebotomy in heparinized tubes, placed on ice, layered over Ficoll Paque density gradients, and centrifuged at 1800 rpm for 20 minutes. The top plasma layers were collected and frozen at -80 °C in preparation for enzyme-linked immunosorbent assay (ELISA) and lymphocyte fractions were separated for mRNA analysis as described below.

Plasma HO-1 protein measurement. Purified recombinant rat HO-1 (SPP-730) and rabbit-derived polyclonal antisera raised against rat HO-1 (SPA-895) were purchased from StressGen Biotechnologies (Victoria, BC, Canada). The latter recognizes human HO-1 and was used previously by our laboratory to demonstrate HO-1 protein overexpression in AD brain by immunohistochemistry and Western blotting.²² Competitive ELISA for the detection and quantification of HO-1 protein was performed on all 175 patients according to the procedure of Wang et al.²⁶ with the following modifications: Immulon II ELISA plates (Dyex Technologies, Chantilly, VA) were coated (50 μ L/well) with 0.5 μ g/mL HO-1 protein (dissolved in coating buffer containing Na₂CO₃ and NaHCO₃ pH 9.6) and incubated overnight at 4 °C. Excess protein was decanted and washed with washing buffer containing Tris, NaCl, and Tween-20. Wells were blocked with 3% bovine serum albumin (BSA) in coating buffer and incubated for 2 hours at 37 °C. Seventy-five microliters of plasma was mixed with 75 μ L of HO-1 antibody (diluted 1/50,000) and incubated for 2 hours at room temperature. After blocking and washing, the plasma and HO-1 antibody mixture was added to each well for overnight incubation at 4 °C. Secondary antibody consisting of alkaline phosphate-conjugated antirabbit immunoglobulin G (IgG) (diluted 1/1000) in 0.1% BSA was added to the wells and incubated for 2 hours at 37 °C. Fifty microliters of the substrate (P-nitrophenyl phosphate

Table Subject characteristics

Diagnosis	n	Age, y (mean)	Sex, F/M	MMSE (mean)	Education, y (mean)
Controls	31	62.5	17/14	29.4	14.1
Age 40–60 y (NYC)	7	47.1	1/6	30	16.6
Age 60+ y (NEC)	24	77.9	16/8	28.9	11.7
AACD	25	76.3	15/10	26.9	10.5
AD	50	76.8	27/23	21.1	10.9
NAD	16	74.3	6/10	11.5	
LBD	4	71.25	2/2	18.2	—
CBGD	2	69.6	1/1	7.5	—
HD	1	63	0/1	24	—
NPH	2	81.0	0/2	25	—
MID	4	77.5	1/3	6.2	—
MSA	1	86	0/1	11	—
Down syndrome	1	55	1/0	0	—
HT	1	86	1/0	0	—
PD	21	68.7	10/11	29.0	—
ALS	14	55.7	8/6	28.9	—
Sporadic	13	56.1	8/5	28.8	—
Familial	1	50	0/1	30	—
Stroke	5	61.8	1/4	26	—
RD	8	63.4	6/2	27.6	—
Liver disease	5	51.8	2/3	27.2	—
HS	4	51.0	2/2	26.5	—
CAH	1	55	0/1	30	—

MMSE = Mini-Mental State Examination; NEC = normal elderly controls; NYC = normal younger controls; AACD = age-associated cognitive decline; NAD = non-Alzheimer dementia; LBD = Lewy body dementia; CBGD = corticobasal ganglionic degeneration; HD = Huntington disease; NPH = normal pressure hydrocephalus; MID = multi-infarct dementia; MSA = multisystem atrophy; HT = head trauma; RD = rheumatic diseases; HS = hepatic steatosis; CAH = chronic active hepatitis (A/B).

disodium dissolved in diethanolamine, pH 9.8) was added to each well and incubated for 20 minutes at room temperature. The resulting color reaction was read at 405 nm on a multiwell scanning spectrophotometer (Molecular Devices Corp., Sunnyvale, CA). HO-1 protein concentrations were determined from a standard curve generated from samples containing varying concentrations (0.01 to 15.0 $\mu\text{g}/\text{mL}$) of purified HO-1 protein and HO-1 antibody (1/50,000). All assays were performed in duplicate.

Lymphocyte HO-1 mRNA measurement. HO-1 mRNA levels were measured by Northern analysis on 162 of the 175 patients (50 AD, 25 AACD, 24 NEC, 7 younger controls, 16 non-Alzheimer dementia, 13 PD, 12 ALS, 5 stroke, 5 liver disease, and 5 rheumatologic disease). Lymphocyte fractions were obtained by differential centrifugation of whole blood on Ficoll Paque gradients. Lymphocyte mRNA was isolated using an acid guanidinium thiocyanate-phenol-chloroform extraction method.²⁷ Six micrograms of RNA was denatured and size-separated by electrophoresis on 1% agarose/formaldehyde gels. RNA integrity was confirmed by ethidium bromide staining. The RNA was transferred onto Hybond-N (Amersham, Oakville, Ontario) filter paper and covalently crosslinked to the membrane by ultraviolet light for 2 minutes. The hybridization probe (full-length human HO-1 cDNA [*Xho*I-*Eco*RI], 1 kb, in pBluescript SKII+ (Gibco, Burlington, On-

tario); a gift from Dr. S. Shibahara, Freidrich-Miescher-Institut, Basel) was prepared using the Random Primer DNA Labeling System (Roche Diagnostics, Laval, Quebec).²⁸ Hybridization was performed under stringent conditions using ³²P-labeled denatured DNA probe²⁹ as previously described.²¹ Equal loading of RNA was confirmed by hybridization with a cDNA for the (housekeeping) gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The RNA hybridizing with the cDNA probes was visualized by autoradiography using an intensifying screen at $-80\text{ }^{\circ}\text{C}$.³⁰ The RNA levels were quantified using a PhosphorImager S1 densitometer. Densitometry data were normalized by calculating the ratios of the HO-1 mRNA signals to control GAPDH signals and the results expressed in arbitrary units.

CSF HO-1 protein concentrations. Frozen ventricular CSF specimens derived postmortem from pathologically proven AD cases (n = 24) and neurohistologically normal controls (n = 12) were obtained from the Institute for Brain Aging and Dementia Brain Tissue Repository (University of California, Irvine). MMSE scores were available for 17 AD patients (mean 11.1 ± 1.9). The *APOE* $\epsilon 4/4$ genotype was present in three AD and no control cases. One $\epsilon 4$ allele was present in 10 AD and no control cases. CSF HO-1 protein concentrations were measured by ELISA as described above. All ELISAs and Northern anal-

yses were performed on coded samples by personnel unaware of patient diagnoses.

Statistics. Statistical analysis of the demographic, MMSE, and blood HO-1 data was performed using one-way analysis of variance (ANOVA) with $p < 0.05$ indicating significance. Fisher PLSD test was applied to determine main effects within groups. CSF HO-1 data were analyzed using unpaired Student's *t*-test (two-tailed) with $p < 0.05$ indicating significance. Correlations between HO-1 data and MMSE scores were assessed using the Pearson correlation coefficient with $p < 0.05$ indicating significance. Optimal sensitivity and specificity of HO-1 mRNA measurement for the diagnosis of AD were determined by receiver operating characteristic (ROC) curve analysis.

Results. Demographic data. There were no significant age differences among the AD, AACD, and NEC cases ($p > 0.05$ for each comparison). Years of formal education in the AD group did not differ significantly from the AACD and NEC cohorts ($p > 0.05$). The AACD group had significantly fewer years of education than the NEC group ($p < 0.02$). MMSE scores were significantly lower in the AD group than in the AACD or NEC cases ($p < 0.001$ for each comparison). Differences in MMSE scores between the AACD and NEC groups were not significant ($p > 0.05$; see the table).

Plasma HO-1 protein levels. Interassay variability inherent to the ELISA, determined by running samples from the same AD and NEC cases on multiple occasions, was approximately 20%. Mean plasma HO-1 protein concentrations were significantly lower ($p < 0.05$) in patients with probable AD ($0.85 \pm 0.14 \mu\text{g/mL}$) in comparison with the NEC (1.77 ± 0.34), stroke (2.55 ± 1.33), rheumatologic disease (1.88 ± 0.5), and liver disease (2.24 ± 1.19) groups (figure 1). Plasma HO-1 levels in the AD patients did not differ significantly ($p > 0.05$) from those of the non-Alzheimer dementia (1.22 ± 0.14), PD (1.25 ± 0.20), and ALS (1.13 ± 0.25) groups. The AACD group exhibited plasma HO-1 concentrations ($1.06 \pm 0.33 \mu\text{g/mL}$) intermediate between, but not statistically different from, those of the AD and NEC cases ($p > 0.05$ for each comparison). In the AD group, there was no significant correlation between plasma HO-1 levels and MMSE scores ($r = 0.16$; $p = 0.27$).

Lymphocyte HO-1 mRNA levels. Interassay variability for the Northern analyses, determined by including lymphocyte RNA samples from several AD and NEC individuals in multiple runs, was 10 to 11%. Lymphocyte HO-1 mRNA levels were lower in the AD cohort than in the NEC ($p < 0.001$), AACD, non-Alzheimer dementia, PD, ALS, stroke, rheumatologic disease, and liver disease groups (figure 2). The NEC values were greater than those of the AACD and non-Alzheimer dementia groups ($p < 0.05$ for each comparison), and similar to those of the younger controls, PD, ALS, stroke, liver disease, and rheumatologic disease groups ($p > 0.05$ for each comparison). In comparison with all groups excluding AACD, the sensitivity and specificity of lymphocyte HO-1 mRNA measurement for the diagnosis of early sporadic AD at a cutoff value of 0.78 arbitrary units (AU) are 88% and 75%. There were no significant correlations between lymphocyte HO-1 mRNA levels and MMSE scores ($r = -0.06$; $p = 0.73$) or between

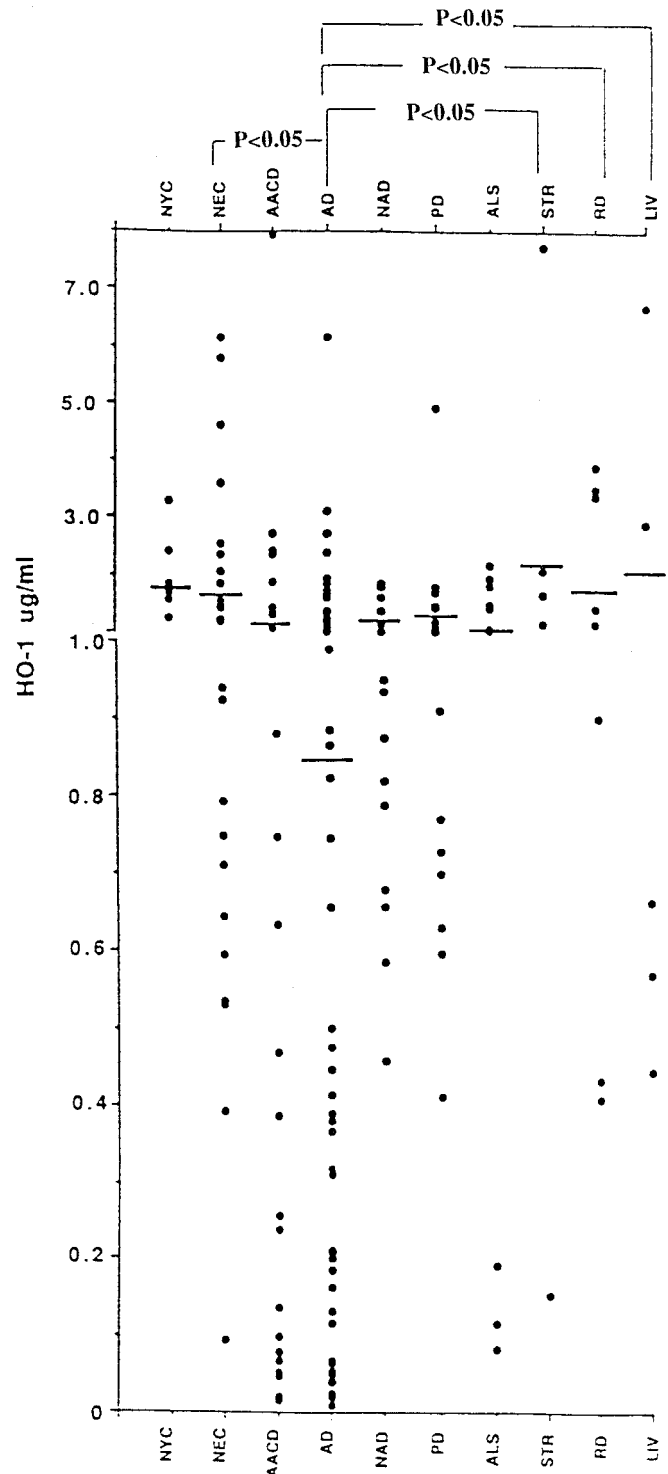
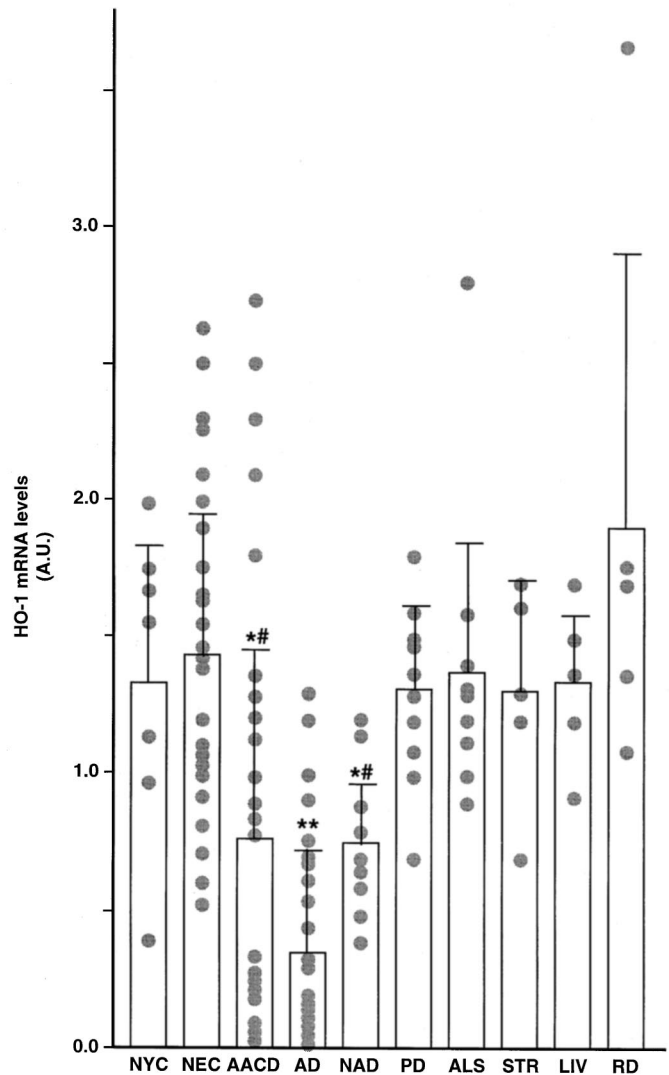
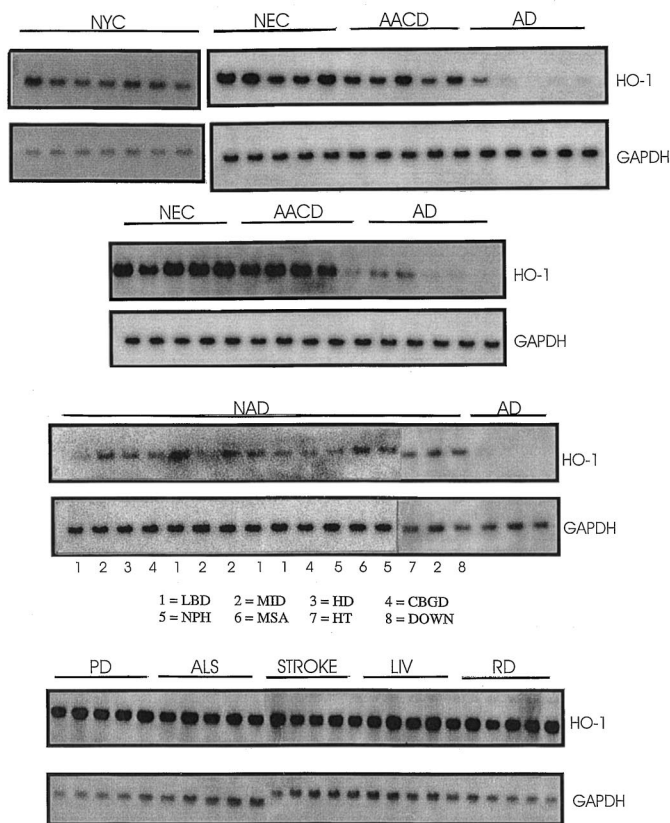


Figure 1. Plasma heme oxygenase-1 (HO-1) protein concentrations (measured by enzyme-linked immunosorbent assay) in all cases described in the table. Horizontal lines denote mean values and statistically significant differences between groups are indicated at the top of the figure. NYC = normal younger controls; NEC = normal elderly controls; AACD = age-associated cognitive decline; NAD = non-Alzheimer dementia; STR = stroke; RD = rheumatic diseases; LIV = liver diseases.



A

B

Figure 2. (A) Representative heme oxygenase-1 (HO-1) mRNA bands (determined by Northern analysis) in lymphocytes derived from study cases. Control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) bands used to ensure uniformity of RNA loading are depicted below the HO-1 bands. NYC = normal younger controls; NEC = normal elderly controls; AACD = age-associated cognitive decline; NAD = non-Alzheimer dementia; LBD = Lewy body disease; MID = multi-infarct dementia; HD = Huntington disease; CBGD = corticobasal ganglionic degeneration; NPH = normal pressure hydrocephalus; MSA = multiple system atrophy; HT = head trauma; LIV = liver diseases; RD = rheumatic diseases. (B) Lymphocyte HO-1 mRNA levels from 162 of the cases enrolled in this study (NYC = 7, NEC = 24, AACD = 25, AD = 50, NAD = 16, PD = 13, ALS = 12, stroke [STR] = 5, LIV = 5, RD = 5). Densitometric data were normalized for control GAPDH expression and are presented in arbitrary units (AU). Columns and vertical lines denote means and standard deviations. * $p < 0.05$ relative to NEC, ** $p < 0.001$ relative to NEC, # $p < 0.01$ relative to AD.

plasma HO-1 protein and lymphocyte HO-1 mRNA levels in the AD and NEC groups ($r = -0.04$; $p = 0.85$).

CSF HO-1 protein levels. Mean CSF HO-1 protein concentrations in cases of pathologically proven AD (19.07 ng/mL) were lower ($p < 0.01$) than those of control cases (32.48 ng/mL; figure 3). CSF HO-1 concentrations showed no significant correlations with MMSE scores ($p = 0.57$) or APOE $\epsilon 4$ genotype ($p = 0.56$) in the AD group.

Discussion. Although immunoreactive HO-1 has previously been detected in circulating lymphocytes,³¹ the presence of HO-1 in human plasma has not, to our knowledge, been previously reported.

HO-1 may contribute to the antioxidant activity of plasma by degrading hemoproteins or free heme to bile pigments (biliverdin, bilirubin) with free radical scavenging capabilities.²⁰ Alternatively, plasma HO-1 may have no biologic function and merely represent "leakage" of the enzyme from tissues to the plasma compartment analogous to the presence of circulating liver enzymes and the iron-storage protein, ferritin.³²

HO-1 is markedly overexpressed in neurons and astrocytes of AD-diseased cerebral cortex and hippocampus relative to age-matched controls.²² Conversely, the results of the current study indicate that

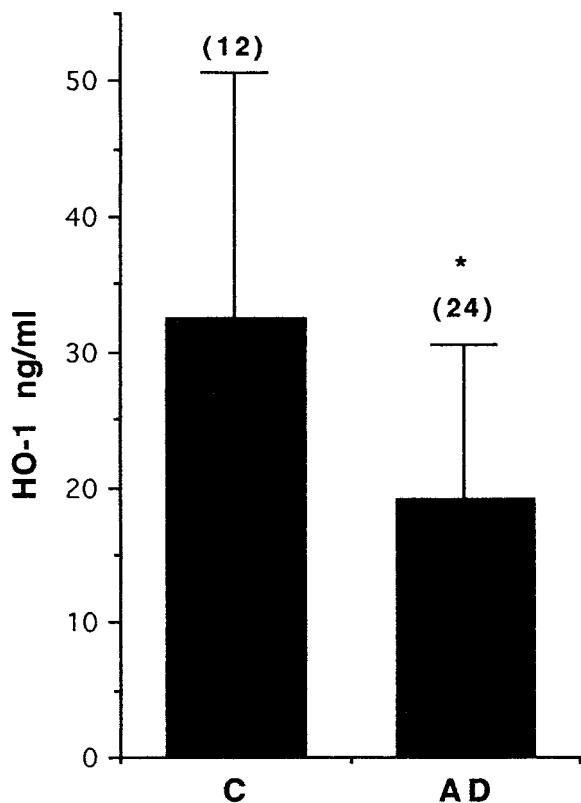


Figure 3. Mean heme oxygenase-1 (HO-1) protein levels (determined by enzyme-linked immunosorbent assay) in CSF derived from neurohistologically normal elderly subjects (C) and pathologically confirmed cases of AD. Columns and vertical lines denote means and standard deviations. * $p < 0.05$ relative to C; number of patients in parentheses.

plasma HO-1 protein levels are significantly *decreased* in patients with probable sporadic AD relative to NEC. In addition, CSF HO-1 protein concentrations in cases of pathologically proven AD are significantly lower than those of neurohistologically normal controls. Plasma HO-1 levels in the AACD group were intermediate between those of the NEC and AD values although these differences were not statistically significant. Lymphocyte HO-1 mRNA levels in the AD patients were markedly suppressed relative to NEC and patients with non-AD dementia, other neurologic conditions, and certain chronic medical disorders. Thus, diminished plasma HO-1 levels in AD probably reflect decreased production of the enzyme as opposed to accelerated degradation of HO-1 in the circulation. In both the AD and NEC groups, there was no apparent correlation between lymphocyte HO-1 mRNA levels and plasma HO-1 protein levels. This may be due to the possibility that plasma HO-1 concentrations reflect the release of HO-1 from multiple tissue sources aside from lymphocytes. Dysregulation of blood HO-1 in AD suggests that systemic redox homeostasis and iron metabolism are perturbed in this condition. Consistent with this notion are reports of altered plasma antioxidant en-

zyme profiles³³ and increased levels of the iron-binding protein p97¹⁷ in AD patients.

Although the upregulation of HO-1 in AD brain can be readily understood as a response to local oxidative stress, the mechanism responsible for the downregulation of HO-1 in the blood of AD patients remains unclear. Kimpara et al.³⁴ did not find overrepresentation of specific HO-1 gene polymorphisms in patients with AD, mitigating against the possibility that primary genetic determinants of HO-1 expression are responsible for the diminished peripheral HO-1 mRNA and protein levels in sporadic AD. Because glucocorticoids are the only known suppressors of HO-1 gene transcription,³⁵ and hypercortisolism and dexamethasone resistance occur in approximately 50% of AD patients,³⁶ we measured serum cortisol concentrations in the same blood samples used for the determination of plasma HO-1 levels in subsets of AD, AACD, and NEC cases. In no group was there an apparent correlation between plasma HO-1 levels and serum cortisol concentrations, suggesting that hypercortisolism is not responsible for peripheral HO-1 suppression in sporadic AD (authors' unpublished results, 1998).

In this phase 2 evaluation,³⁷ lymphocyte HO-1 mRNA levels exhibited high sensitivity and moderate to high specificity in differentiating patients with probable AD from NEC and patients with other neurologic and medical disorders. These data implicate HO-1 as a systemic biologic marker in sporadic AD. As in the majority of AD cases, the lymphocyte HO-1 mRNA bands in several patients with suspected Lewy body disease and multi-infarct dementia were less intense than the weakest bands observed in the NEC. The former may in fact represent additional cases of AD because a significant proportion of subjects with these conditions are found to harbor neuropathologic changes typical of AD at autopsy.³⁸ In light of this consideration, and because in the current study individuals with non-Alzheimer dementia exhibited more severe cognitive impairment than the AD cohort, further evaluation of blood HO-1 levels in the various dementing illnesses will be required. Lymphocyte HO-1 mRNA levels in patients with PD, ALS, cerebral infarctions, chronic liver disease, and rheumatologic disorders did not differ significantly from those of healthy controls.

Plasma HO-1 concentrations and lymphocyte HO-1 mRNA levels in individuals with AACD were intermediate between those of the AD and NEC cohorts, suggesting that HO-1 gene suppression in peripheral tissues is a very early event in the natural history of sporadic AD. Cases of AACD/MCI pose a major diagnostic dilemma inasmuch as about half of these individuals will progress to AD over the ensuing 3 years,³⁹ whereas an important subgroup will manifest no further cognitive decline over long-term follow-up.⁸ Longitudinal assessment of the AACD cohort with serial cognitive evaluations and blood HO-1 measurements should determine whether AACD/MCI cases with the lowest blood HO-1 levels

are at higher risk for the development of incipient AD than are neuropsychologically identical AACD/MCI cases with blood HO-1 levels in the high normal range.

HO-1 gene expression in peripheral lymphocytes may satisfy certain criteria for a useful biologic marker in sporadic AD, as recently defined in a Consensus Report sponsored by the Ronald and Nancy Reagan Research Institute (Alzheimer's Association) and National Institute on Aging^{4,40}: 1) Diminished lymphocyte HO-1 mRNA levels exhibit high sensitivity and moderate to high specificity in differentiating sporadic AD from normal controls and patients with various dementing and nondementing neurologic and medical disorders. 2) Lymphocyte HO-1 mRNA levels are suppressed in early stages of AD and in some individuals with AACD. 3) Although the mechanism responsible for the downregulation of HO-1 in the blood of AD patients remains unclear, blood HO-1 abnormalities may reflect events occurring in the disease process because a) HO-1 is highly overexpressed and colocalizes to senile plaques and neurofibrillary tangles in AD brain,^{22,23} and b) central upregulation of HO-1 may promote aberrant iron deposition and mitochondrial lesions characteristic of AD-affected neural tissues.²¹ 4) Lymphocyte HO-1 assays are relatively noninvasive and inexpensive, and could be readily available in many hospital laboratories. Testing of several thousand individuals (a phase 3 study) and independent confirmation of the findings by at least one other laboratory will be necessary to substantiate diminished blood HO-1 levels as a diagnostically useful marker of sporadic AD.^{9,37} Further work will also be required to assess blood HO-1 protein and mRNA levels in autopsy-proven cases of sporadic (and familial) AD and to elucidate the mechanisms of peripheral HO-1 gene suppression in this common neurodegenerative disorder.

Acknowledgment

The authors thank Adrienne Liberman, Marlene Levine, Line Beaudet, and Andrew Feifer for excellent technical help and nursing assistance; Drs. Nora Kelner and Lennie Babins for neuropsychological testing; Shelley Solomon, Chris Hosein, and the medical staff of the McGill Memory Clinic for help with AD patient recruitment; Dr. Jeffrey Minuk and the medical and nursing staff of the Maimonides Hospital Geriatric Center and the ALS Association of Quebec for recruitment of patients with stroke, non-Alzheimer dementia, and ALS; the Institute for Brain Aging and Dementia Brain Tissue Repository (University of California, Irvine) for CSF specimens; Victor Whitehead for assistance with data analysis; and Rhona Rosenzweig and Renée Kaminski for skillful secretarial support.

References

1. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984;34:939-944.
2. Galasko D, Hansen L, Katzman R, et al. Clinical-neuropathological correlations in Alzheimer's disease and related dementias. *Arch Neurol* 1994;51:888-895.
3. Gearing M, Mirra SS, Hedreen JC, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part

- X. Neuropathology confirmation of the clinical diagnosis of Alzheimer's disease. *Neurology* 1995;45:461-466.
4. Kennard M. Diagnostic markers for Alzheimer's disease. *Neurobiol Aging* 1998;19:131-132.
5. Levy R. Aging-associated cognitive decline. Working party of the International Psychogeriatric Association in collaboration with the World Health Organization. *Int Psychogeriatr* 1994; 6:63-68.
6. Rubin EH, Morris JC, Grant E, Vendegna T. Very mild senile dementia of the Alzheimer type. I. Clinical assessment. *Arch Neurol* 1989;46:379-382.
7. Chertkow H, Bergman H, Wolfson C, Babins L, Kelner N. Standard neuropsychological tests do not predict development of Alzheimer's disease in individuals with "age-associated cognitive decline." *Can J Neurol Sci* 1998;25(suppl 1):S27-S28.
8. Dawe B, Procter A, Philpot M. Concepts of mild memory impairment in the elderly and their relationship to dementia—a review. *Int J Geriatr Psychiatry* 1992;7:473-479.
9. Trojanowski JQ, Growdon JH. A new consensus report on biomarkers for the early antemortem diagnosis of Alzheimer disease: current status, relevance to drug discovery, and recommendations for future research. *J Neuropathol Exp Neurol* 1998;57:643-644.
10. St. George-Hyslop PH. Role of genetics in tests of genotype, status, and disease progression in early-onset Alzheimer's disease. *Neurobiol Aging* 1998;19:133-137.
11. Hyman BT. Biomarkers in Alzheimer's disease. *Neurobiol Aging* 1998;19:159-160.
12. Motter R, Vigo-Pelfrey C, Kholodenko D, et al. Reduction of beta-amyloid peptide 42 in the cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol* 1995;38:643-648.
13. Ida N, Hartmann T, Pantel J, et al. Analysis of heterogeneous betaA4 peptides in human cerebrospinal fluid and blood by a newly developed sensitive Western blot assay. *J Biol Chem* 1996;271:22908-22914.
14. Hock C, Golombowski S, Naser W, et al. Increased levels of tau protein in cerebrospinal fluid of patients with Alzheimer's disease—Correlation with cognitive impairment. *Ann Neurol* 1995;37:414-415.
15. de la Monte SM, Volicer L, Hauser SL, et al. Increased levels of neuronal thread protein in cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol* 1992;32:733-742.
16. Iwatsubo T. Amyloid beta protein in plasma as a diagnostic marker for Alzheimer's disease. *Neurobiol Aging* 1998;19:161-163.
17. Kennard ML, Feldman H, Yamada T, Jefferies WA. Serum levels of the iron binding protein p97 are elevated in Alzheimer's disease. *Nat Med* 1996;2:1230-1235.
18. Feldman H. Diagnosis of Alzheimer's disease. Presented at the annual meeting of the Royal College of Physicians & Surgeons (Canada); Vancouver; September 25-28, 1997.
19. Tenhunen R, Marver HS, Schmid R. Microsomal heme oxygenase: characterization of the enzyme. *J Biol Chem* 1969; 244:6388-6394.
20. Stocker R, Yamamoto Y, McDonagh AF, et al. Bilirubin is an antioxidant of possible physiological importance. *Science* 1987;235:1043-1046.
21. Schipper HM, Bernier L, Mehindate K, et al. Mitochondrial iron sequestration in dopamine-challenged astroglia: role of HO-1 and the permeability transition pore. *J Neurochem* 1999;72:1802-1811.
22. Schipper HM, Cissé S, Stopa EG. Expression of heme oxygenase-1 in the senescent and Alzheimer-diseased brain. *Ann Neurol* 1995;37:758-768.
23. Smith MA, Kutty RK, Richey PL, et al. Heme oxygenase-1 is associated with the neurofibrillary pathology of Alzheimer's disease. *Am J Pathol* 1994;145:42-47.
24. Folstein M, Folstein F, McHugh P. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *J Clin Res* 1975;12:189-198.
25. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of dementia. *Br J Psychiatry* 1982;140:566-572.
26. Wang GP, Iqbal K, Ducht G, Winblad B, Wisniewski HM, Grundke-Iqbal I. Alzheimer's disease: paired helical filament immunoreactivity in cerebrospinal fluid. *Acta Neuropathol* 1991;82:6-12.
27. Chomczynski P, Sacchi N. Single-step method of RNA isolation.

- tion by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analyt Biochem* 1987;162:156–159.
28. Feinberg AP, Vogelstein B. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Analyt Biochem* 1984;137:266–267.
 29. Noonberg SB, Scott GK, Hunt CA, Benz CC. Detection of triplex-forming RNA oligonucleotides by triplex blotting. *Bio-Techniques* 1994;16:1070–1072.
 30. Church GM, Gilbert W. Genomic sequencing. *Proc Natl Acad Sci USA* 1984;81:1991–1995.
 31. Menzel DB, Rasmussen RE, Lee E, et al. Human lymphocyte heme oxygenase-1 as a response biomarker to inorganic arsenic. *Biochem Biophys Res Commun* 1998;250:653–656.
 32. Ponka P, Beaumont C, Richardson DR. Function and regulation of transferrin and ferritin. *Sem Hematol* 1998;35:35–54.
 33. Famulari AL, Marschoff ER, Llesuy SF, et al. The antioxidant enzymatic blood profile in Alzheimer's and vascular diseases. Their association and a possible assay to differentiate demented subjects and controls. *J Neurol Sci* 1996;141:69–78.
 34. Kimpura T, Takeda A, Watanabe K, et al. Microsatellite polymorphism in the human heme oxygenase-1 gene promoter and its application in association studies with Alzheimer and Parkinson disease. *Hum Genet* 1997;100:145–147.
 35. Abraham NG, Drummond GS, Lutton JD, Kappas A. The biological significance and physiological role of heme oxygenase. *Cell Physiol Biochem* 1996;6:129–168.
 36. Balldin J, Gottfries C, Karlsson I, Lindstedt G, Langstrom G, Walinder J. Dexamethasone suppression test and serum prolactin in dementia disorders. *Br J Psychiatr* 1983;143:277–281.
 37. Litvan I. Methodological and research issues in the evaluation of biological diagnostic markers for Alzheimer's disease. *Neurobiol Aging* 1998;19:121–123.
 38. Liebson E, Albert ML. Cognitive changes in dementia of the Alzheimer type. In: Calne DB, ed. *Neurodegenerative diseases*. Philadelphia: WB Saunders, 1994:615–629.
 39. Thal L. Potential prevention strategies for Alzheimer's disease. *Alzheimer Dis Assoc Disord* 1996;10(suppl 1):6–8.
 40. Klunk WE. Biological markers of Alzheimer's disease. *Neurobiol Aging* 1998;19:145–147.

MRI volumetric study of dementia with Lewy bodies

A comparison with AD and vascular dementia

R. Barber, MRCPsych; C. Ballard, MD; I.G. McKeith, MD; A. Gholkar, FRCR; and J.T. O'Brien, DM

Article abstract—*Objective:* To compare global and regional atrophy on MRI in subjects with dementia with Lewy bodies (DLB), AD, vascular dementia (VaD), and normal aging. In addition, the relationship between *APOE-ε4* genotype and volumetric indices was examined. *Method:* MRI-based volume measurements of the whole-brain, ventricles, frontal lobe, temporal lobe, hippocampus, and amygdala were acquired in elderly subjects with DLB ($n = 27$; mean age = 75.9 years), AD ($n = 25$; 77.2 years), VaD ($n = 24$; 76.9 years), and normal control subjects ($n = 26$; 76.2 years). *Results:* Subjects with DLB had significantly larger temporal lobe, hippocampal, and amygdala volumes than those with AD. No significant volumetric difference between subjects with DLB and VaD was observed. Compared with control subjects, ventricular volumes were increased in all patients with dementia, though those with DLB showed a relative preservation of whole-brain volume. There were no significant differences in frontal lobe volumes between the four groups. *APOE-ε4* status was not associated with volumetric indices. *Conclusion:* The findings support the hypothesis that DLB is associated with a relative preservation of temporal lobe structures. In the differentiation of DLB and AD, this may have important implications for diagnosis. **Key words:** Dementia with Lewy bodies—Alzheimer's disease—Vascular dementia—MRI—Temporal lobe—Frontal lobe.

NEUROLOGY 2000;54:1304–1309

Dementia with Lewy bodies (DLB) is characterized by fluctuating cognitive impairment, persistent visual hallucinations, and parkinsonism. It is the second most common form of degenerative dementia and may account for up to 20% of cases of late-life dementia.¹ Ante mortem diagnosis of DLB and its differentiation from other common forms of late-onset dementias, particularly AD and vascular dementia (VaD), is important for a number of reasons. Some

patients with DLB may have an accelerated disease progression and approximately 50% of subjects experience life-threatening adverse reactions to antipsychotic medications (neuroleptic sensitivity).² In contrast, subjects with DLB may have an enhanced therapeutic response to treatment with cholinesterase inhibitors.³

Volumetric MRI may provide important supplementary information that could support or counter a

From the Institute for the Health of the Elderly (Drs. Barber, Ballard, McKeith, and O'Brien), and the Department of Neuroradiology (Dr. Gholkar), Newcastle General Hospital, Newcastle upon Tyne, UK.

Supported by a grant from the Northern and Yorkshire Regional Health Authority and Medical Research Council, UK.

Received August 9, 1999. Accepted in final form December 3, 1999.

Address correspondence and reprint requests to Dr. R. Barber, Institute for the Health of the Elderly, Westgate Road, Newcastle General Hospital, Newcastle upon Tyne, NE4 6BE, UK; e-mail: robert.barber@ncl.ac.uk