

Plasma levels of A β peptides are altered in amnesic mild cognitive impairment but not in sporadic Alzheimer's disease

Tomasz Sobów¹, Marcin Flirski¹, Iwona Kłoszewska¹, and
Paweł P. Liberski²

¹Department of Old Age Psychiatry and Psychotic Disorders; ²Department of Molecular Pathology and Neuropathology, Medical University of Lodz, 8/10 Czechoslowacka St., 92-216 Lodz, Poland

Abstract. Plasma A β levels have been examined in sporadic Alzheimer's disease yielding conflicting results; both no difference and an increase in plasma concentrations of A β_{42} and A β_{40} in sporadic cases of AD as compared to controls have been reported. Elevated plasma A β_{42} levels may be detected several years before the onset of symptoms (in mild cognitive impairment stadium). Levels of A β_{40} and A β_{42} were measured in plasma from 54 patients with AD, 39 subjects with MCI and 35 controls using a commercially available ELISA. Mean plasma A β_{42} levels were significantly higher in MCI as compared to both AD ($P < 0.001$) and control subjects ($P < 0.001$), while A β_{40} did not differ between the groups. No correlations were observed between A β levels and age, MMSE scores or gender. According to ROC curve analysis the maximum accuracy in discriminating MCI *versus* both controls and AD subjects has been achieved using a cut-off value of 3.8.

The correspondence should be addressed to T. Sobów, Email: tmsobow@csk.umed.lodz.pl

Key words: amyloid-beta protein, plasma, Alzheimer's disease, mild cognitive impairment

INTRODUCTION

Dementia is one of the strongest predictors of mortality, with a risk two to three times those of other life-shortening illnesses, thus constituting a major medical and socioeconomic burden (Tschanz et al. 2004). Alzheimer's disease (AD) is the most prevalent cause of dementia in the developed countries, occupying the eighth place among the ten leading causes of death (Fitzpatrick et al. 2004, Minino et al. 2002). A definite diagnosis of sporadic AD can only be obtained owing to brain biopsy or at post-mortem neuropathologic examination. Routine clinical, neuropsychological and neuroimaging diagnostic procedures, while costly and time-consuming, can at best result in a diagnosis of "probable AD", as defined in the DSM-IV and ICD-10 sets of criteria (Knopman et al. 2001). Moreover, in the early stages of the disease, when its presentation may not yet be typical, AD is often misclassified, e.g., as a disorder of the frontotemporal dementia spectrum (Galton et al. 2000). A biological marker providing additional information helpful in the differential diagnosis of AD is therefore mostly required (Sobów et al. 2004).

Mild cognitive impairment (MCI), introduced as a clinical construct in the mid-'90s, is a manifestation of a heterogeneous underlying pathology and characterizes a diverse population of patients, the subgroup of which progress to an overt dementia (Dubois and Albert 2004). Longitudinal studies reveal that a percentage of MCI cases (particularly of the amnesic type) evolve into clinically overt AD, with the annual conversion rates usually estimated at 5–10%, although even exceeding 15% in some studies (Amieva et al. 2004). However, a significant proportion of MCI patients return to the state with no cognitive impairment without any treatment or their cognitive deterioration remains stable (Larrieu et al. 2002, Petersen et al. 1999). It is therefore hardly possible clinically to single out those MCI cases which represent an early stage in the development of AD.

Currently, the most extensively evaluated pathogenetic theory related to AD is the "amyloid cascade hypothesis". According to that theory, deposition of the insoluble, proteolysis-resistant forms of A β peptide subsequently initiates the cascade of events leading to neuronal death and eventually development of the clinical symptoms of AD (Hardy and Higgins 1992). In contrast to familial cases of AD (FAD) that are strong-

ly related to β APP mismetabolism, pathogenesis of the sporadic form of AD is vague. It is probably the convergence of both genetic (e.g. APO E or CYP46 polymorphisms) and nongenetic (e.g., hormonal influence, pathology of microcirculation) factors that synergistically lead to neuropathological and clinical image similar to FAD (Selkoe 2004, Żekanowski et al. 2004).

A β is generated during constitutive cellular metabolism and secreted to the extracellular space, allowing its detection in the CSF (Shoji 2002). Results of the early studies evaluating total A β concentrations in the CSF of AD patients are contradictory, as either lack of change, slight increase or slight decrease have been reported (Andresen and Blennow 2002). However, in the majority of studies a statistically significant decline in the concentration of the 42 amino acid-residue-long isoform of A β (A β_{42}) has consistently been observed in the CSF of AD patients (Andreasen et al. 1999, Motter et al. 1995). This phenomenon can, at least partially, be explained by a diminished clearance of A β_{42} which precipitates into amyloid plaques (Strozyk et al. 2003). The decrease in A β_{42} has even been reported in MCI subjects, thus possibly rendering this marker available in the earliest stages of the disease (Hampel et al. 2004). Concentrations of A β_{42} have been stable longitudinally, correlated neither with severity nor with progression rate of dementia (Andreasen et al. 1999a). The applicability of CSF A β_{42} assessment as a diagnostic marker of AD is narrowed by its low specificity towards other types of dementia, e.g., dementia with Lewy bodies, Creutzfeldt-Jakob disease, vascular dementia (Andreasen et al. 1999b). Furthermore, an important restriction is the necessity of lumbar puncture, a procedure which outside of Scandinavian countries and Japan is carried out relatively rarely owing to reluctance of the patients.

Plasma levels of A β peptides represent a potentially attractive biomarker of AD. They are relatively easy to assess and, importantly, might reflect a central pathogenic process of the disorder, e.g., β APP metabolism deregulation and consecutive brain A β accumulation. Plasma A β_{42} concentrations are elevated in FAD (Kosaka et al. 1997, Scheuner et al. 1996). The results of studies on the population of patients with sporadic AD are inconclusive; either an increase (Mayeux et al. 1999, Mehta et al. 2000) or lack of change (Tamaoka et al. 1996, Vanderstichele et al. 2000) have been reported. It has also been proposed that plasma A β_{42} levels increase merely with age, regardless of the diag-

nostic category (Fukumoto et al. 2003). Finally, elevated plasma A β_{42} concentrations may be observed several years before the onset of symptoms (Graff-Radford et al. 2002, Mayeux et al. 1999), though the results of one study suggest that this effect is restricted to women only (Assini et al. 2004).

The aim of the present study was to assess the levels of A β peptides (A β_{40} and A β_{42}) in plasma of AD patients and MCI subjects compared to healthy controls matched for age, gender and education and to estimate the sensitivity and specificity of plasma A β concentrations as diagnostic markers for AD and amnesic MCI.

METHODS

Patients' evaluation and selection

It's been repetitively shown that a considerable proportion of subjects with other than AD types of dementia tend to fulfill internationally accepted AD criteria, e.g., those of NINCDS-ADRDA working group. Therefore, one of the major obstacles in getting reliable results in AD clinical studies might be patients selection. Here, we attempted to overcome this source of potential bias by using restrictive patients selection method targeted to exclude patients with mixed AD and patients fulfilling diagnostic criteria for other specific forms of dementia, e.g., dementia with Lewy bodies or frontotemporal dementia. Since it is the amnesic form of MCI which is most probably a clinical precursor of AD, only subjects with a preliminary diagnosis of amnesic MCI were recruited. That way we hoped to enrich our sample in "pure" AD cases.

All the patients involved in the study were diagnosed in the specialized university-based Alzheimer's Clinic during the period of 2001–2003. Initially, a total number of 132 newly diagnosed patients fulfilling the NINCDS-ADRDA diagnostic criteria for AD were found eligible. To ensure that our sample is not "contaminated" by cases with high probability of suffering from non-AD or mixed dementia we excluded: 36 patients fulfilling ICD-10 criteria for mixed AD dementia, 11 patients fulfilling diagnostic criteria for dementia with Lewy bodies, 5 subjects fulfilling criteria for frontotemporal dementia, and 8 patients with a long-term history of addiction (or harmful use) to alcohol, benzodiazepines or barbiturates, alone or in combination. As the goal of our study was to evaluate a

diagnostic marker in subjects with sporadic AD, from the remaining cohort of 72 participants we excluded another 18 with a family history of AD. From the preliminarily recruited group of 70 subjects fulfilling Petersen's criteria for MCI we excluded 31 cases for the following reasons: presence of an isolated deficit in a single cognitive domain other than memory ($n=4$), multiple domain deficits ($n=7$), clinically significant or badly controlled cardiovascular disorders, the influence of which on the development of the observed deficits could not be excluded ($n=11$), long-term history of addiction (or harmful use) to alcohol, benzodiazepines or barbiturates, alone or in combination ($n=2$), and family history of AD ($n=7$).

All the participants or their caregivers (in case of AD patients) were provided with detailed information on the study protocol and signed an informed consent form; the study protocol was approved by the Ethical Committee of the Medical University of Lodz.

Sample characteristics and plasma A β levels measurements

Finally, 128 participants were enrolled in the study: 54 patients with sporadic AD (17 men, mean age 77.5 ± 4.4 years, mean MMSE 17.5 ± 3.4), 39 subjects with amnesic MCI (13 men, mean age 74.0 ± 3.4 , mean MMSE 27.3 ± 0.9), and 35 cognitively intact elderly without clinically significant vascular dementia risk factors or family history of dementia (11 men, mean age 75.0 ± 2.9 , mean MMSE 29.5 ± 0.6) (Table I).

Whole blood was collected from fasting subjects in EDTA-containing recipients and cellular material was pelleted by centrifugation. Platelets have been regarded a primary source of circulating β APP and A β . However, no sampling technique modification preventing the activation of platelets was applied, based on data indicating lack of any associations between platelet activation and plasma A β levels measured with a similar method (Olsson et al. 2003). As an increasing number of reports fail to observe a correlation between statins treatment and plasma A β levels, we did not introduce any additional procedures in cases of hypercholesterolemia treated with statins (Hoglund et al. 2004). Plasma was stored at -4°C for a maximum of 8 hours and then frozen in 1 ml aliquots and stored at -70°C until measurements. The concentrations of A β peptides (A β_{40} and A β_{42}) in plasma were measured using commercially available sandwich ELISA colori-

Table I

Demographic characteristics of the study participants							
Study group	<i>n</i>	Age (mean ± SD)	Gender (fraction of women)	Years of formal education (mean ± SD)	Age at symptomatic onset (AD cases only)	MMSE score (mean ± SD)	ADAS-cog score (mean ± SD)
AD	54	77.5 ± 4.4	0.69	7.3 ± 3.3	73.5 ± 4.2	17.5 ± 3.4	30.5 ± 10.6
MCI	39	74.0 ± 3.4	0.66	10.0 ± 3.2	N/A	27.3 ± 0.9	10.4 ± 2.7
Controls	35	75.0 ± 2.9	0.68	8.6 ± 2.9	N/A	29.5 ± 0.6	3.0 ± 1.9
Total	128	75.6 ± 4.1	0.68	8.5 ± 3.3	N/A	23.8 ± 5.9	16.9 ± 13.9

metric assay (BioSource Intl, Inc) which has been shown to be sensitive enough (range 15.6–1 000 pg/ml) to ensure an accurate result in plasma.

RESULTS

Mean plasma concentrations of A β peptides (values in pg/ml ± SD) in AD, MCI and control subjects are presented in Table II; A β_{40} /A β_{42} quotient – by some authors considered more sensitive than A β_{42} levels alone – was calculated as well. The statistical analysis of between-group differences was performed using a nonparametric U Mann-Whitney test; the differences between groups in age, years of formal education and MMSE test scores (see Table I) were adjusted for. No

difference in any of the evaluated parameters (A β_{40} , A β_{42} , A β_{40} /A β_{42}) was observed between the AD and control groups. In subjects with MCI plasma A β_{42} levels were significantly elevated versus both AD ($P < 0.001$) and controls ($P < 0.001$), whereas A β_{40} concentrations were not significantly altered. This pattern of changes resulted in a significantly lower A β_{40} /A β_{42} ratio in the MCI group compared to both AD ($P < 0.001$) and control groups ($P < 0.001$) (Fig. 1). Contrary to some previous reports, a linear regression method analysis (ANOVA) did not reveal any correlation between age of the participants and plasma A β levels; the observed differences were also unrelated to subjects' gender.

Both visual inspection of the boxplots (Fig. 1) and the comparison of means using independent samples nonparametric tests revealed that A β_{40} /A β_{42} ratio and A β_{42} levels alone could almost perfectly separate MCI subjects from both AD and controls. As a next step, we have performed area under the curve ROC analyses to estimate the cut-off values of A β_{42} and A β_{40} /A β_{42} ratio that yield a highest accuracy in discriminating the samples. The value of A β_{40} /A β_{42} ratio lower than 3.8 discriminated MCI from AD with a sensitivity of 97.4% (95% CI = 86.5–99.6) and specificity of 83.3% (70.7–92.1), and MCI from controls with a sensitivity of 97.4% (86.5–99.6) and specificity of 88.6% (73.2–96.7). The level of A β_{42} higher than 45 pg/ml discriminated MCI from AD with a sensitivity of 94.9% (82.6–99.2) and specificity of 75.9% (62.4–86.5), while MCI from controls with a sensitivity of 94.4% (82.6–99.2) and specificity of 91.4% (76.9–98.1). The comparison of ROC curves for the evaluated variables is presented in Figures 4 and 5.

Table II

Mean values of plasma levels of A β peptides (pg/ml ± SD) and A β_{40} /A β_{42} ratio in the study groups				
Study group	<i>n</i>	A β_{40} *	A β_{42} **	A β_{40} /A β_{42} **
AD	54	168.7 ± 32.2	37.8 ± 10.3	4.6 ± 0.9
MCI	39	160.1 ± 20.2	56.8 ± 9.3	2.9 ± 0.6
Controls	35	160.1 ± 15.2	36.3 ± 6.3	4.5 ± 0.6

(*) differences not significant (U Mann-Whitney test);
 (**) differences significant between MCI and both AD and controls at $P < 0.001$ (U Mann-Whitney test)

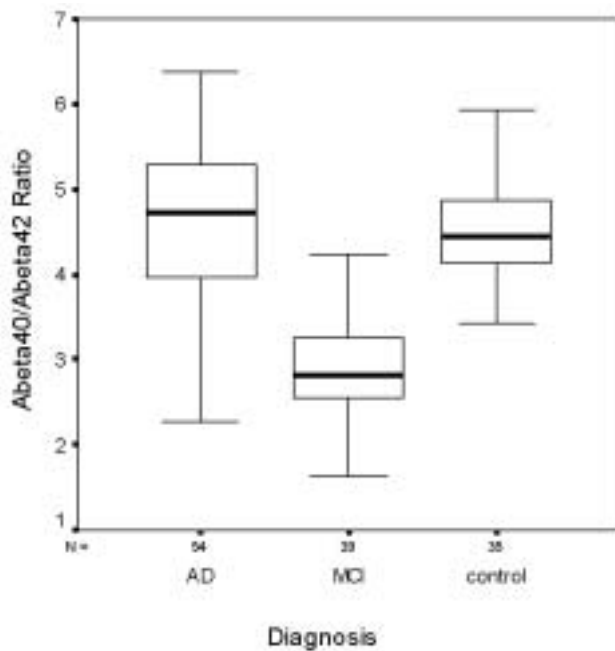


Fig. 1. Boxplots showing differences between the study groups in A β_{40} /A β_{42} ratio. Note that although there is some overlap between the groups, there is close to perfect separation of MCI subjects from both AD and controls considering 95% confidence intervals (grey zones of boxplots).

DISCUSSION

The comparison of concentrations of A β peptides in plasma of AD subjects versus carefully selected control group presented in our study indicates the lack of applicability of this assessment in the differential diagnosis of sporadic AD. The absence of statistically significant differences is in concordance with some of the previous papers (Fukumoto et al. 2003, Tamaoka et al. 1996, Vanderstichele et al. 2000). We did not observe a formerly reported correlation between plasma A β levels and age of the study participants (Fukumoto et al. 2003) or any other demographic variable, like MMSE score, gender or years of formal education (Assini et al. 2004). We found an increase in A β_{42} concentrations in plasma of MCI subjects compared to either AD patients or controls; therefore, alterations in plasma A β_{42} might represent a potentially sensitive biochemical marker in that group of patients (Assini et al. 2004, Graff-Radford et al. 2002, Mayeux et al. 1999).

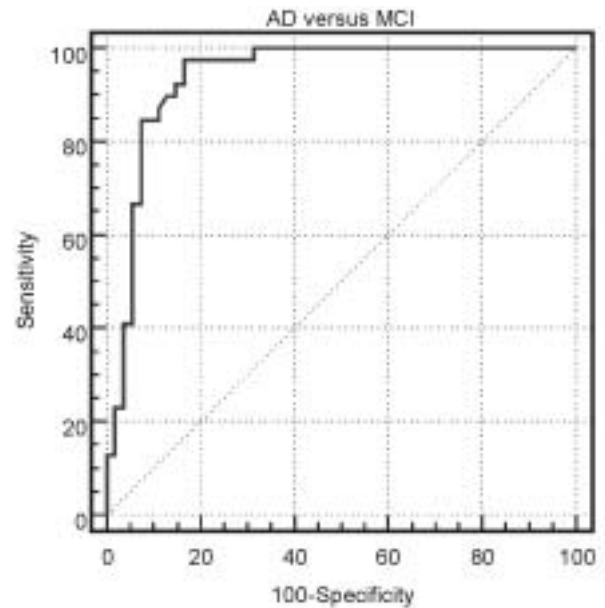


Fig. 2. Receiver Operating Characteristic (ROC) curve for A β_{40} /A β_{42} ratio as a discriminating test for MCI and AD. The value of A β_{40} /A β_{42} ratio lower than 3.8 discriminates MCI from AD with sensitivity of 97.4% (95% CI = 86.5–99.6) and specificity of 83.3% (95% CI = 70.7–92.1); an open box on the curve represents maximum sensitivity/specificity point. Area under the ROC curve = 0.938; SE = 0.025; 95% confidence interval = 0.867 to 0.977.

In an attempt to explain the obtained results, a hypothesis can be formulated that the elevation of plasma A β_{42} in MCI is a reflection of efforts to eliminate the pathological peptide from the central nervous system (CNS) through the yet viable blood-brain barrier (BBB). In the later stages of disease, when the BBB is impaired, elimination of A β_{42} from the brain would become ineffective which might lead to its accumulation, toxicity towards neurons, and progressive cell death. The efficiency of A β peptides' elimination in earlier stages of AD has indirectly been proven on animal models (Das et al. 2001); this mechanism can be responsible for the efficacy of both active and passive immunization in diminishing the number of amyloid deposits in the brains of experimental animals (DeMattos et al. 2001, 2002). Moreover, there is a substantial evidence for a progressive damage of the BBB in the course of neurodegeneration in AD (Claudio 1996, Kalaria 1999). Some authors point out the possibility of functional changes in the BBB associated with passage of A β

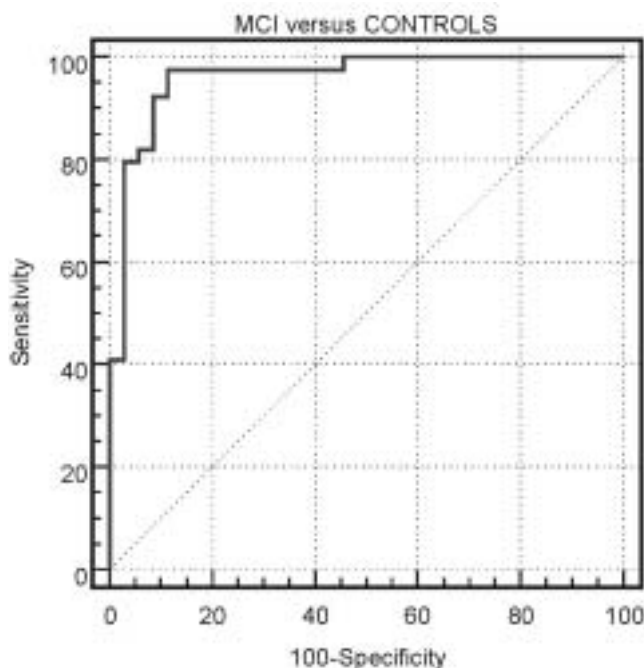


Fig. 3. Receiver Operating Characteristic (ROC) curve for $A\beta_{40}/A\beta_{42}$ ratio as a discriminating test for MCI and controls. The value of $A\beta_{40}/A\beta_{42}$ ratio lower than 3.8 discriminates MCI from controls with sensitivity of 97.4% (95% CI = 86.5–99.6) and specificity of 88.6% (73.2–96.7); an open box on the curve represents maximum sensitivity/specificity point. Area under the ROC curve = 0.961; SE = 0.024; 95% confidence interval = 0.888 to 0.992.

peptides and their putative indirect influence on its permeability (Pluta et al. 1996, Shibata et al. 2000, Strazielle et al. 2000). It is therefore probable that in a very early stage of AD alterations in the catabolism of β APP occur, resulting in a preferential synthesis of longer, more amyloidogenic isoform of $A\beta$ peptide – $A\beta_{42}$; $A\beta_{42}$ is initially effectively removed *via* the BBB (by means of mechanisms associated with the LRP-1 receptor and proteins influencing lipid metabolism, such as $\alpha 2$ -macroglobulin and apolipoprotein E) which brings about a gradual increase in plasma $A\beta_{42}$, but not $A\beta_{40}$, still competently transported backwards (Shibata et al. 2000, Strazielle et al. 2000).

The results of our study, indicating an elevation of $A\beta_{42}$, but not $A\beta_{40}$ in MCI subjects, would support this hypothesis. In later stages of AD, owing to a progressive destruction of the BBB and changes in a brain/plasma amyloid balance, both failure in the removal of $A\beta_{42}$ from the brain (and its increased dep-

osition in amyloid plaques) and retrograde transport of $A\beta$ peptides from plasma to the brain (mainly responsible for vascular amyloidosis) would occur (Bading et al. 2002, Mackic et al. 2002). With this theoretical sequence of events the lack of changes in plasma concentrations of $A\beta$ peptides can be explained.

CONCLUSIONS

The plasma level of $A\beta_{42}$ or $A\beta_{40}/A\beta_{42}$ quotient allow – with around 95% sensitivity and at least 75% specificity – the discrimination of MCI subjects from healthy controls and AD patients; unfortunately, that does not necessarily impose that a subgroup with “progressive” MCI (evolving towards AD) can be singled out with these parameters. For this purpose, a long-term follow-up is inevitable; we have initiated it on the described study group. It is also possible that the elevation of plasma $A\beta_{42}$ will constitute a personal indicator of the risk of conversion into AD rather than classical biomarker of the trait.

REFERENCES

- Amieva H, Letenneur L, Dartigues JF, Rouch-Leroyer I, Sourgen C, D'Alchee-Biree F, Dib M, Barberger-Gateau P, Orgogozo JM, Fabrigoule C (2004) Annual rate and predictors of conversion to dementia in subjects presenting mild cognitive impairment criteria defined according to a population-based study. *Dement Geriatr Cogn Disord* 18: 87–93.
- Andreasen N, Blennow K (2002) β -amyloid ($A\beta$) protein in cerebrospinal fluid as a biomarker for Alzheimer's disease. *Peptides* 23: 1205–1214.
- Andreasen N, Hesse C, Davidsson P, Minthon L, Wallin A, Winblad B, Vanderstichele H, Vanmechelen E, Blennow K (1999a) Cerebrospinal fluid β -amyloid (1–42) in Alzheimer disease: differences between early- and late-onset Alzheimer disease and stability during the course of disease. *Arch Neurol* 56: 673–680.
- Andreasen N, Minthon L, Clarberg A, Davidsson P, Gottfries J, Vanmechelen E, Vanderstichele H, Winblad B, Blennow K (1999b) Sensitivity, specificity, and stability of CSF-tau in AD in a community-based patient sample. *Neurology* 57: 1488–1494.
- Assini A, Cammarata S, Vitali A, Colucci M, Giliberto L, Borghi R, Inglese ML, Volpe S, Ratto S, Dagna-Bricarelli F, Baldo C, Argusti A, Odetti P, Piccini A, Tabaton M (2004) Plasma levels of amyloid beta-protein 42 are

- increased in women with mild cognitive impairment. *Neurology* 63: 828–831.
- Bading JR, Yamada S, Mackic JB, Kirkman L, Miller C, Calero M, Ghiso J, Frangione B, Zlokovic BV (2002) Brain clearance of Alzheimer's amyloid-beta40 in the squirrel monkey: A SPECT study in a primate model of cerebral amyloid angiopathy. *J Drug Target* 10: 359–368.
- Claudio L (1996) Ultrastructural features of the blood-brain barrier in biopsy tissue from Alzheimer's disease patients. *Acta Neuropathol (Berl)* 91: 6–14.
- Das P, Murphy MP, Younkin LH, Younkin SG, Golde TE (2001) Reduced effectiveness of Abeta1-42 immunization in APP transgenic mice with significant amyloid deposition. *Neurobiol Aging* 22: 721–727.
- DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM (2001) Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* 98: 8850–8855.
- DeMattos RB, Bales KR, Cummins DJ, Paul SM, Holtzman DM (2002) Brain to plasma amyloid-beta efflux: A measure of brain amyloid burden in a mouse model of Alzheimer's disease. *Science* 295: 2264–2267.
- Dubois B, Albert ML (2004) Amnestic MCI or prodromal Alzheimer's disease? *Lancet Neurol* 3: 246–248.
- Fitzpatrick AL, Kuller LH, Ives DG, Lopez OL, Jagust W, Breitner JC, Jones B, Lyketsos C, Dulberg C (2004) Incidence and prevalence of dementia in the Cardiovascular Health Study. *J Am Geriatr Soc* 52: 195–204.
- Fukumoto H, Tennis M, Locascio JJ, Hyman BT, Growdon JH, Irizarry MC (2003) Age but not diagnosis is the main predictor of plasma amyloid beta-protein levels. *Arch Neurol* 60: 958–964.
- Galton CJ, Patterson K, Xuereb JH, Hodges JR (2000) Atypical and typical presentations of Alzheimer's disease: A clinical, neuropsychological, neuroimaging and pathological study of 13 cases. *Brain* 123: 484–498.
- Graff-Radford N, Ertekin-Taner N, Jadeja N, Younkin L, Younkin S (2002) Evidence that plasma amyloid beta protein may be useful as a premorbid biomarker for Alzheimer's disease. *Neurobiol Aging* 23: S384.
- Hampel H, Teipel SJ, Fuchsberger T, Andreasen N, Wiltfang J, Otto M, Shen Y, Dodel R, Du Y, Farlow M, Moller HJ, Blennow K, Buerger K (2004) Value of CSF beta-amyloid1-42 and tau as predictors of Alzheimer's disease in patients with mild cognitive impairment. *Mol Psychiatry* 9: 705–710.
- Hardy JA, Higgins GA (1992) Alzheimer's disease: The amyloid cascade hypothesis. *Science* 256: 184–185.
- Hoglund K, Wiklund O, Vanderstichele H, Eikenberg O, Vanmechelen E, Blennow K (2004) Plasma levels of beta-amyloid(1-40), beta-amyloid(1-42), and total beta-amyloid remain unaffected in adult patients with hypercholesterolemia after treatment with statins. *Arch Neurol* 61: 333–337.
- Kalaria RN (1999) The blood-brain barrier and cerebrovascular pathology in Alzheimer's disease. *Ann N Y Acad Sci* 893: 113–125.
- Knopman DS, DeKosky ST, Cummings JL, Chui H, Corey-Bloom J, Relkin N, Small GW, Miller B, Stevens JC (2001) Practice parameter: diagnosis of dementia (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 56: 1143–1153.
- Kosaka T, Imagawa M, Seki K, Arai H, Sasaki H, Tsuji S, Asami-Odaka A, Fukushima T, Imai K, Iwatsubo T (1997) The beta APP717 Alzheimer mutation increases the percentage of plasma amyloid-beta protein ending at A beta42(43). *Neurology* 48: 741–745.
- Larrieu S, Letenneur L, Orgogozo JM, Fabrigoule C, Amieva H, Le Carret N, Barberger-Gateau P, Dartigues JF (2002) Incidence and outcome of mild cognitive impairment in a population-based prospective cohort. *Neurology* 59: 1594–1599.
- Mackic JB, Bading J, Ghiso J, Walker L, Wisniewski T, Frangione B, Zlokovic BV (2002) Circulating amyloid-beta peptide crosses the blood-brain barrier in aged monkeys and contributes to Alzheimer's disease lesions. *Vascul Pharmacol* 38: 303–313.
- Mayeux R, Tang MX, Jacobs DM, Manly J, Bell K, Merchant C, Small SA, Stern Y, Wisniewski HM, Mehta PD (1999) Plasma amyloid beta-peptide 1-42 and incipient Alzheimer's disease. *Ann Neurol* 46: 412–416.
- Mehta PD, Pirttila T, Mehta SP, Sersen EA, Aisen PS, Wisniewski HM (2000) Plasma and cerebrospinal fluid levels of amyloid beta proteins 1-40 and 1-42 in Alzheimer disease. *Arch Neurol* 57: 100–105.
- Minino AM, Arias E, Kochanek KD, Murphy SL, Smith BL (2002) Deaths: Final data for 2000. *Natl Vital Stat Rep* 50: 1–119.
- Motter R, Vigo-Pelfrey C, Kholodenko D, Barbour R, Johnson-Wood K, Galasko D, Chang L, Miller B, Clark C, Green R (1995) Reduction of β -amyloid peptide 42 in the cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol* 38: 643–648.
- Olsson A, Vanmechelen E, Vanderstichele H, Davidsson P, Blennow K (2003) Unaltered plasma levels of beta-amy-

- loid(1-40) and beta-amyloid(1-42) upon stimulation of human platelets. *Dement Geriatr Cogn Disord* 16: 93-97.
- Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E (1999) Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol* 56: 303-308.
- Pluta R, Barcikowska M, Januszewski S, Misicka A, Lipkowski AW (1996) Evidence of blood-brain barrier permeability/leakage for circulating human Alzheimer's beta-amyloid-(1-42)-peptide. *Neuroreport* 7: 1261-1265.
- Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med* 2: 864-870.
- Selkoe DJ (2004) Alzheimer disease: mechanistic understanding predicts novel therapies. *Ann Intern Med* 140: 627-638.
- Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, Holtzman DM, Miller CA, Strickland DK, Ghiso J, Zlokovic BV (2000) Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J Clin Invest* 106: 1489-1499.
- Shoji M (2002) Cerebrospinal fluid Abeta40 and Abeta42: Natural course and clinical usefulness. *Front Biosci* 7: 997-1006.
- Sobów T, Flirski M, Liberski PP (2004) Amyloid-beta and tau proteins as biochemical markers of Alzheimer's disease. *Acta Neurobiol Exp (Wars)* 64: 53-70.
- Strazielle N, Ghersi-Egea JF, Ghiso J, Dehouck MP, Frangione B, Patlak C, Fenstermacher J, Gorevic P (2000) In vitro evidence that beta-amyloid peptide 1-40 diffuses across the blood-brain barrier and affects its permeability. *J Neuropathol Exp Neurol* 59: 29-38.
- Strozyk D, Blennow K, White LR, Launer LJ (2003) CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology* 60: 652-656.
- Tamaoka A, Fukushima T, Sawamura N, Ishikawa K, Oguni E, Komatsuzaki Y, Shoji S (1996) Amyloid beta protein in plasma from patients with sporadic Alzheimer's disease. *J Neurol Sci* 141:65-68.
- Tschanz JT, Corcoran C, Skoog I, Khachaturian AS, Herrick J, Hayden KM, Welsh-Bohmer KA, Calvert T, Norton MC, Zandi P, Breitner JC; Cache County Study Group (2004) Dementia: The leading predictor of death in a defined elderly population: The Cache County Study. *Neurology* 62: 1156-1162.
- Vanderstichele H, Van Kerschaver E, Hesse C, Davidsson P, Andreasen N, Minthon L, Wallin A, Blennow K, Vanmechelen E (2000) Standardization of measurement of beta-amyloid(1-42) in cerebrospinal fluid and plasma. *Amyloid* 7: 245-258.
- Żekanowski C, Religa D, Graff C, Filipek S, Kuźnicki J (2004) Genetic aspects of Alzheimer's disease. *Acta Neurobiol Exp* 64 (Wars): 19-31.

Received 14 April 2005, accepted 21 April 2005