Abnormalities in the Pattern of Platelet Amyloid Precursor Protein Forms in Patients With Mild Cognitive Impairment and Alzheimer Disease

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Context: Patients affected by sporadic Alzheimer disease (AD) show a significant alteration of amyloid precursor protein (APP) forms in platelets when compared with patients with dementia but without AD and age-matched controls.

Objective: To evaluate the ratio of platelet APP forms (APPr) in early-stage AD and mild cognitive impairment (MCI) and its potential as a biomarker for the early identification of AD.

Setting: Community population-based sample of patients admitted to 4 AD centers for investigation of cognitive disturbances.

Design and Methods: Thirty-five patients with mild AD (mAD), 21 patients with very mild AD (vmAD), 30 subjects with MCI, and 25 age-matched controls were included. The APPr was evaluated by Western blot analysis in platelet homogenate.

Results: Compared with controls (mean±SD, 0.93±0.3), the mean APPr was decreased in patients with mAD (0.44±0.24; P<.001) and patients with vmAD (0.49±0.3; P<.001). Regarding the MCI group, a significant decrease in APPr was found compared with controls (0.62±0.33; P<.001). Fixing a cutoff score of 0.6, sensitivity was 88.6% (31/35) for patients with mAD and 85.7% (18/21) for patients with vmAD, whereas specificity was 88% (22/25) for controls. Among patients with MCI, 18 (60%) of 30 individuals displayed APPr values below the cutoff.

Conclusions: Alteration of platelet APP forms is an early event in AD, and the measurement of APPr may be useful for the identification of preclinical AD in patients with MCI.

Arch Neurol. 2002;59:71-75

Alzheimer disease (AD) is a chronic neurodegenerative disorder characterized by a progressive cognitive and memory decline. It is defined by senile plaques, neurofibrillary tangles, and amyloid angiopathy, neuropathologic hallmarks that allow for a definitive diagnosis of the disease.1,2 In fact, pathologically confirmed preclinical AD occurs before the development of cognitive impairment,3 suggesting that AD lesions must be present for a sufficient length of time to produce neuronal or synaptic loss.4 On the other hand, there is evidence that in most cases the disease progresses to the stage of dementia after only several years from the appearance of the first behavioral symptoms.5-7

In this regard, great attention has been devoted to the transitional stage of cognitive impairment between normal aging and early AD, so-called mild cognitive impairment (MCI).6,8 Mild cognitive impairment refers to the clinical state of individuals who are memory impaired but are otherwise functioning well and do not meet clinical criteria for dementia.10-12 There is evidence that individuals with MCI are at an increased risk for developing AD, and several investigators13-15 strongly believe that virtually all patients with MCI have neuropathologic AD. Nevertheless, proposed criteria for MCI may well apply to a heterogeneous population affected by drug-induced states, affective disorders, or systemic diseases. Early detection is, therefore, vitally important, but at present the diagnostic process requires an extensive follow-up study based on clinical and laboratory examinations.16,17

It would be desirable to have a biomarker that helps identify patients affected by AD at the so-called predementia stage when pharmacologic effects on cognitive functioning and symptom progression could be most beneficial. In the past few years, different biochemical parameters in cerebral spinal fluid (CSF) or plasma...
PATIENTS AND METHODS

PATIENTS

Participants were recruited from different neurologic and geriatric centers in Italy (Clinica Neurologica, Università degli Studi di Brescia; Centro Alzheimer, Ospedale di Rho; Dipartimento Scienze Neurologiche, Università “La Sapienza”; Istituto di Ricerca a Carattere Cura Scientifica, Santa Lucia) using a standardized diagnostic protocol. The study was conducted in accordance with local clinical research regulations, and informed consent was required from all subjects and caregivers when indicated.

All subjects performed a somatic and neurologic examination. The following laboratory studies were performed on the sample population: routine examination, complete blood cell count, sedimentation rate, vitamin B₁₂ and folic acid measurement, thyroid hormone dosage measurement, syphilis serologic testing, and homocysteine measurement. All patients underwent a brain imaging study (computed tomography or magnetic resonance imaging).

The behavioral and global cognitive evaluation was conducted according to a standardized battery, which included the following tools: Clinical Dementia Rating scale (CDR), Mini-Mental Status Examination (MMSE), Alzheimer Disease Assessment Scale, Neuropsychiatric Inventory, Geriatric Depression Scale, Hamilton Anxiety Rating Scale, instrumental activities of daily living, and activities of daily living. The neuropsychologic testing was accomplished by the following tests: Raven Coloured Progressive Matrices, Controlled Oral Word Association Test, Category Fluency (animal names), Clock Drawing Test, Rey Complex Figure Copy and Recall, Story Recall Test, and Trail-Making Test.

The diagnosis of MCI was based on Mayo Clinic criteria: (1) subjective memory complaint, (2) normal activities of daily living, (3) normal general cognitive functioning, (4) abnormal verbal and/or nonverbal memory for age, and (5) absence of dementia. The diagnosis of dementia and probable AD was based on Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) and on National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association criteria, respectively. Patients with AD entered this study only after being drug free for at least 14 days before blood collection and platelet preparation.

PLATELET COLLECTION AND PREPARATION

Blood samples were obtained from fasting participants between 9 AM and 10 AM. A blood sample (27 mL) was drawn, carefully releasing the tourniquet immediately after its application, from a vein in the antecubital fossa using a 19-gauge needle, and collected into 3 mL of 3.8% sodium citrate (in the presence of 2450 mg/dL [136 mmol/L] of glucose). Each sample was mixed gently and centrifuged at 200 ×g for 10 minutes to separate platelet-rich plasma within 30 minutes after blood drawing. Platelet-rich plasma was separated from the blood pellet by means of a plastic pipette, avoiding aspiration of the bulky coat. Platelets were then collected by further centrifugation at 3000g for 20 minutes. The platelet pellet was stored at −80°C. Immunoblot experiments were performed with monoclonal antibody 22C11 as described elsewhere. Patient information and case diagnosis were unknown to the laboratory investigators who received and analyzed the blood samples.

STATISTICAL ANALYSIS

Comparisons among groups was performed using factor analysis of variance with post hoc analyses (Tukey honestly significant difference test for unequal sample sizes). Sensitivity (ie, the proportion of patients with AD and low APPr) and specificity (ie, the proportion of controls with high APPr) were calculated using a cutoff score (normal APPr, >0.6) from a study performed on a large sample of patients with AD and control individuals (data not shown).

have been investigated, and several studies have shown that tau levels or a combination of tau and β-amyloid levels in CSF might increase the discrimination of AD and may predict the development of AD in patients with MCI.

Recently, different authors have tried to identify peripheral markers of AD, focusing mostly on the amyloid precursor protein (APP), a protein expressed ubiquitously in several splice variants, which is believed to play a key role in the pathogenesis of AD through the formation of the Aβ peptide.

In this regard, it has been observed that patients affected by sporadic AD show a significant alteration of the immunoreactivity of the APP forms in platelets when compared with patients with dementia but without AD and age-matched controls, suggesting that the measurement of the ratio of APP forms (APPr) may represent a reliable and sensitive marker for AD.
These observations define the frame of the present study, which aims to investigate whether APP changes in platelets occur at the earliest clinically detectable stage of AD. More specifically, we addressed the following questions: (1) Is the platelet APPr decreased in patients who fulfill criteria for MCI, very mild AD (vmAD), and mild AD (mAD) compared with age-matched controls? (2) How accurate is the APPr in discriminating among mAD, vmAD, and age-matched controls? (3) What proportion of patients with MCI show alterations of APP forms in platelets?

The analysis was performed on 111 blood samples from individuals 55 to 90 years old. The groups consisted of 35 patients diagnosed as having mAD, 21 patients with vmAD, 30 subjects with MCI, and 25 age-matched controls. Demographic and clinical characteristics of the study population are given in Table 1.

Whole platelet homogenates from each patient and control were processed for Western blot analysis by means of monoclonal antibody 22C11 raised against the N-terminal domain of APP, thereby recognizing all APP forms. Thereafter, the optical density of the bands at 106, 110, and 130 kd was measured by image analysis; the APPr was determined.

### RESULTS

Comparison between platelet APPr levels among the 4 subgroups are presented in Table 2. There was a significant decrease in the level of APPr in the mAD group compared with the control group (P<.001). An APPr decrease was also found in the vmAD group (P<.001) compared with controls. No significant differences were found between the vmAD group and the mAD group.

With regard to the MCI group, a significant decrease in APPr values was found compared with the control group (P<.001). There was no significant difference between the MCI and both the vmAD and the mAD groups, although in the MCI group, there was higher variability and greater overlapping of APPr values with the control group (Figure 1).

Sensitivity and specificity data for APPr using the cutoff score are presented in Figure 2. Sensitivity was 88.6% (32/35) for mAD and 85.7% (18/21) for vmAD, whereas specificity was 88% (22/25) for the control group. By using this cutoff score, 18 (60%) of 30 patients with MCI displayed APPr values below the cutoff.

### COMMENT

Our results demonstrate that alteration in APP metabolism is an early event in sporadic AD and that most patients with MCI have prodromal AD according to the platelet APPr. These findings extend previous reports and confirm that APP metabolic changes are widespread, involving central and peripheral tissues early in the disease.

The mechanisms that lead to platelet APP alterations and their relation to the pathologic changes typically found in the AD brain are still poorly understood. Although unlikely to contribute to cerebral amyloid deposition, platelet-associated APP forms provide an appropriate tissue to study APP biochemistry and metabolism in both healthy and diseased patients. Further, the findings of the present study agree with other AD-related platelet features, such as abnormal activation, increased membrane fluidity, alterations of phospholipases (A and C), and protein kinase C levels.

According to these observations, platelets have been proposed as a source of human biological material that mirrors, in the peripheral compartment, the occurrence and evolution of AD-related biochemical processes that develop in the central nervous system. Moreover, on the basis of the results derived from different clinical settings which showed that the sensitivity and specificity of the measurement of platelet APPr for AD was high, it has been claimed that this assay holds the potential to be a clinical marker, though not a single definitive test.
that MCI actually represents early-stage AD. Nevertheless, not every elderly individual with memory deficits progresses to AD dementia, and there is evidence from postmortem studies that supports other non-AD causes of memory decline. Further, MCI has also been related to several etiologic factors, such as affective disorders, drug-induced states, infarcts, system diseases, and other concomitant diseases, including blood pressure disturbances.3,14,58 Our data partially support the concept that MCI refers to a heterogeneous population, although the relatively large number of patients with MCI and APPr abnormalities is consistent with the view that these patients are at high risk for AD, if not prodromal AD, and that AD can be identified at earlier stages.

In our opinion, these values seem promising for clinical usefulness in the prediction of AD, a longitudinal study on MCI and vmAD has been already planned. Our study has some limitations. First, follow-up of the patients or neuropathological data to confirm the diagnosis are not presently available. We carefully devoted much attention on the diagnostic issue by using widely established standardized criteria to minimize the risk of diagnostic errors. Nevertheless, we agree that studies with patients with neuropathologically confirmed disease are needed to determine the real predictive value of platelet APPr for AD. Second, as with any hospital-based sample, there may be selection biases in our groups, particularly among the patients with MCI. According to inclusion and exclusion criteria, this study was restricted to individuals with minimal confounding comorbidity and pharmacologic treatment, limiting the number of ambiguous cases as much as possible. This might have determined an overestimation of the accuracy measures. On the other hand, some exclusion criteria are still required to avoid interfering or confounding factors on the physiology of platelets and, therefore, on the reliability of the assay. Finally, the study was based on a relatively small sample size, and follow-up is lacking. In this regard, other authors29 have reported very similar findings on APPr in patients with AD, suggesting that the measurement of APPr might be useful to monitor the disease. Furthermore, to determine the real clinical usefulness in the prediction of AD, a longitudinal study on MCI and vmAD has been already planned.

In conclusion, the results of the present study suggest that low platelet APPr is already found in early-phase AD and MCI. These findings may have a role in the diagnostic workup of patients with MCI, identifying patients who will likely develop AD. This will be especially important because in this phase drugs may have the greatest potential of improving symptoms or slowing the disease process.

Accepted for publication September 7, 2001.

Author Contributions: Study concept and design (Drs Padovani, Borroni, Trabucchi, Cattabeni, and Di Luca); acquisition of data (Drs Padovani, Pettenati, Cottini, and Agosti); analysis and interpretation of data (Drs Padovani, Colciaghi, Lenzi, Caltagirone, and Di Luca); drafting of the manuscript (Drs Padovani, Borroni, Colciaghi, Cottini, and Di Luca); critical revision of the manuscript for important intellectual content (Drs Pettenati, Agosti, Lenzi, Caltagirone, Trabucchi, and Cattabeni); obtained funding (Drs Padovani, Lenzi, and Cattabeni); administrative, technical, and material support (Dr Borroni); study supervision (Drs Caltagirone and Di Luca).
This study was supported by grants 9906158271-004 (Dr Di Luca) and 9906158271-003 (Dr Padovani) from MURST 40% and from “Progetto Finalizzato Alzheimer” Ministero della Salute.

We thank Luca Rozzini, MD, Paola Corsini, MD, and Barbara Vicini, MD, for participation in patient enrollment and Roberto Monastero, MD, for helpful discussion and editorial review. We also thank the subjects who donated their time to take part in the study and the staff of the Unità di Neuropatologia degli Spedali Civili of Brescia for accommodating the study.

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REFERENCES