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.

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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. In fact, pathologically confirmed preclinical AD occurs before the development of cognitive impairment, suggesting that AD lesions must be present for a sufficient length of time to produce neuronal or synaptic loss. 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.

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). 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. There is evidence that individuals with MCI are at an increased risk for developing AD, and several investigators 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.

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

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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 $B_{12}$ and folic acid measurement, thyroid hormone dosing measurement, sphyllis 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 if their MMSE score was 19 or 30. Higher patients were grouped as having mAD (CDR score, 0.5) or mAD (CDR score, 1).

The following exclusion criteria were designed for probable AD as the cause of their dementia: (1) major depressive disorder, bipolar disorder, schizophrenia, substance use disorder, or mental retardation according to criteria of DSM-IV; (2) cerebrovascular disorders, hydrocephalus, and intracranial mass, documented by computed tomography or magnetic resonance imaging within the past 12 months; (3) abnormalities in serum folate and vitamin $B_{12}$ levels, sphyllis serologic test results, or thyroid hormone levels; (4) a history of traumatic brain injury or other neurologic disease (eg, Parkinson disease, Huntington disease, seizure disorders); and (5) considerable medical problems (eg, poorly controlled diabetes or hypertension; cancer within the past 5 years; clinically significant hepatic, renal, cardiac, or pulmonary disorders). All these criteria were also adopted for patients with MCI and the control group. The control group comprised 2 subgroups: healthy individuals (including healthy volunteers, spouses, or caregivers) and patients without dementia (including patients with stroke, myelopathies, and head trauma). To avoid potential pharmacologic confounding effects on platelet physiologic findings, patients taking psychotropic agents, nootropic drugs, cholinergic or anticholinergic agents, antipatelelet agents, anticoagulants, corticosteroids, or serotoninergic drugs entered the 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 200g 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 buffy 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).
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?

### Results

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.

Table 1. Demographic and Clinical Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls</th>
<th>MCI Patients</th>
<th>vmAD Patients</th>
<th>mAD Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>25</td>
<td>30</td>
<td>21</td>
<td>35</td>
</tr>
<tr>
<td>Age, mean ± SD, y</td>
<td>69.8 ± 9.0</td>
<td>69.6 ± 6.9</td>
<td>66.2 ± 6.9</td>
<td>69.2 ± 8.5</td>
</tr>
<tr>
<td>Sex, F/M</td>
<td>11/14</td>
<td>17/13</td>
<td>12/9</td>
<td>23/12</td>
</tr>
<tr>
<td>MMSE score, mean ± SD</td>
<td>29.4 ± 1.0</td>
<td>27.9 ± 1.2</td>
<td>24.9 ± 0.9</td>
<td>20.0 ± 1.8</td>
</tr>
</tbody>
</table>

*APPr indicates amyloid precursor protein ratio; MCI, mild cognitive impairment; vmAD, very mild Alzheimer disease; mAD, mild Alzheimer disease; and MMSE, Mini-Mental State Examination (corrected for age and education). P<.001 for mAD patients vs controls, mAD patients vs MCI patients, mAD patients vs vmAD patients, vmAD patients vs MCI patients. P<.01 for MCI patients vs controls.

Table 2. APPr in the Different Groups

<table>
<thead>
<tr>
<th>APPr</th>
<th>Controls</th>
<th>MCI Patients</th>
<th>vmAD Patients</th>
<th>mAD Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>0.93 (0.30)</td>
<td>0.62 (0.33)</td>
<td>0.49 (0.30)</td>
<td>0.44 (0.24)</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.78-1.07</td>
<td>0.49-0.74</td>
<td>0.36-0.63</td>
<td>0.36-0.53</td>
</tr>
</tbody>
</table>

*APPr indicates amyloid precursor protein ratio; MCI, mild cognitive impairment; vmAD, very mild Alzheimer disease; mAD, mild Alzheimer disease; and CI, confidence interval. P<.001 for mAD patients vs controls, vmAD patients vs controls, and MCI patients vs controls. P=.16 (nonsignificant) for mAD patients vs MCI patients. P=.98 (nonsignificant) for MAD patients vs VMAD patients. P=.67 (nonsignificant) for mAD patients vs MCI patients.

### 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.
AD in patients with MCI. Similarly, the present study more interestingly, high sensitivity for the prediction of logical studies and suggest that changes in platelet APP criteria for MCI. These data agree with recent neuropathological studies and already be detectable when cognitive impairment is taking place. 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.

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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. 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.

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 authors 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.

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REFERENCES