Risk Factors for Stroke as Predictors of Platelet Membrane Fluidity in Alzheimer’s Disease

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We have previously reported that increased platelet membrane fluidity identifies a subgroup of patients with Alzheimer’s disease who have distinct clinical features including an earlier age of symptomatic onset, a more rapidly progressive cognitive decline, and a decreased prevalence of focal electroencephalographic findings. In the current study, these patients also exhibited a decreased prevalence of risk factors for stroke compared with patients who had normal platelet membrane fluidity. Our findings suggest that the platelet membrane abnormality describes a clinical subgroup of patients with Alzheimer’s disease who are less likely to have coexisting cerebrovascular disease than the remaining patients who meet clinical consensus criteria for probable Alzheimer’s disease. (Stroke 1991;22:997-1003)

A number of abnormalities have been observed in nonneural cells from patients with Alzheimer’s disease, several of which reflect an alteration in the structure or function of cell membranes.1-4 Among these abnormalities is an increase in platelet membrane fluidity as measured by the fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) in labeled membranes.5 This finding has been observed in controlled clinical studies conducted at three sites by two independent groups of investigators.5-8 At the cellular level, multiple lines of evidence suggest that the increase in platelet membrane fluidity associated with Alzheimer’s disease results from an aberration of an internal membrane compartment resembling smooth endoplasmic reticulum.4,6-11 Abnormalities of cell membrane composition12-14 and structure15-17 have also been found in brain tissue from patients with histologically confirmed Alzheimer’s disease. Of interest, these studies include suggestive evidence of an impairment in endoplasmic reticulum from both the neocortex and allocortex that may be related to the formation of neurofibrillary tangles.18

The increase in platelet membrane fluidity in Alzheimer’s disease appears to be relatively specific for this late-life mental disorder.3,4,6,19 Increased membrane fluidity was not observed in platelets from patients with multi-infarct dementia,6 major depression,3 or mania19 or from patients with Parkinson’s disease who were cognitively intact.4 A cutoff point for DPH anisotropy of 0.1920 at 37°C (the 90th percentile for healthy elderly subjects) separates patients with Alzheimer’s disease into two clinical subtypes.6-8 As a group, patients with increased platelet membrane fluidity (DPH anisotropy of <0.1920) have an earlier onset of cognitive impairment,3 a more rapidly progressive course,3 and a decreased prevalence of focal electroencephalographic (EEG) abnormalities.20 A family history of dementia also appears to be a more common feature of patients in this group.3,21

Longitudinal assessments have indicated that increased platelet membrane fluidity is a relatively stable characteristic.22 Moreover, this membrane phenotype is present at increased prevalence among asymptomatic first-degree relatives of probands with Alzheimer’s disease who manifest increased platelet membrane fluidity8 and predicts an earlier onset of cognitive impairment in those first-degree relatives who develop primary dementia.21 In a segregation analysis employing data from 95 individuals from 14 pedigrees, at least 80% of the variation in platelet membrane fluidity could be explained by the inheritance of a single major locus.23 These data suggest that the platelet membrane abnormality antedates...
the onset of symptoms in this subgroup of patients with Alzheimer’s disease and provides a conceptual framework for understanding how an abnormality in a peripheral tissue may be relevant to the pathophysiology of a central neurodegenerative disorder.

The focus of the current study is to explore the basis for the decreased prevalence of focal EEG findings in the subgroup of patients with Alzheimer’s disease who have increased platelet membrane fluidity. Although the interpretation of focal EEG slowing in the elderly is controversial, one possibility is that at least a fraction of these cases results from focal ischemic changes.24-28 If this hypothesis is responsible for the nonrandom distribution of focal EEG findings between the two fluidity subtypes, patients with Alzheimer’s disease who manifest increased platelet membrane fluidity would be expected to include fewer cases with cerebral ischemia. To test this hypothesis, we determined whether established risk factors for atherothrombotic brain infarction predicted the platelet membrane fluidity subtype of patients with Alzheimer’s disease. Since platelets have been implicated in the pathogenesis of stroke,29,30 it also seemed possible that an alteration of platelet membrane fluidity might affect the risk of stroke in the general population. Therefore, we also examined the relation of platelet membrane fluidity to established risk factors for stroke in a group of neurologically healthy elderly controls.

**Subjects and Methods**

The study population consisted of 49 demented subjects who met National Institute for Neurological and Communicative Disorders and Stroke—Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) consensus clinical criteria for probable Alzheimer’s disease31 and 105 neurologically healthy elderly controls. The 49 demented patients participated in a previously published study of EEG correlates of increased platelet membrane fluidity in Alzheimer’s disease.20 The elderly controls were recruited from advertisements in the media and were typically spouses or caregivers of patients in the Alzheimer’s disease group.

After written informed consent was obtained, a complete history and physical examination, including detailed neurological, psychiatric, and mental status examinations, were performed for patients and controls as previously described.32-34 All subjects had a normal blood count, urinalysis, blood chemistry screen, serum folate and B12 levels, and thyroid function tests, and a Hachinski ischemic score of ≤4.35 None was suffering from malnutrition or vitamin deficiency syndromes as determined by clinical and laboratory evaluations. The computed tomograms (CT scans) of all patients were normal for age or showed cerebral atrophy. The clinical diagnosis of probable Alzheimer’s disease was made conjointly by a psychiatrist and a neurologist on the basis of the insidious onset of dementia with progression in the absence of other systemic or brain diseases that may cause dementia. At our center, the clinical diagnosis of probable Alzheimer’s disease using NINCDS-ADRDA criteria is confirmed by histological evidence in approximately 85% of cases that are autopsied.36

Subjects with disorders that affect blood cell membrane lipid composition or serum lipid profiles, as well as subjects who were on restrictive diets, were excluded from the study.3,10 Individuals who had any history of treatment with phenothiazine neuroleptics, or who were currently being treated with any medication known to affect platelet membrane fluidity by in vitro or in vivo exposure, were excluded from these two groups.3,15,27 In addition, all patients remained free of any drug ingestion, including the use of aspirin, for at least 10 days prior to blood drawing.

Published studies have found the variables in Table 1 to be risk factors for atherothrombotic brain infarction.38 The index of cardiac disease was a composite measure that ranged from a minimum of 0 to a maxi-
mum of 9 and included 1 point each for a history of myocardial infarction or rheumatic heart disease, angina, cardiac pacemaker, heart murmur, symptomatic postural hypotension, orthopnea, peripheral edema secondary to congestive heart failure, and significant electrocardiographic abnormalities. The degree of cognitive impairment was graded by use of the Mini-Mental State Examination (0, worst; 30, best).39

Blood drawing and blood cell isolations were performed according to a minor modification of the method of Corash and coworkers,40 as previously described.3,10 Anticoagulated blood samples were coded by the clinical staff before transport to the laboratory to ensure that all blood processing and subsequent analyses would be carried out by personnel who were blinded to clinical or other laboratory data. Final platelet yields were >90% in all cases. Platelet preparations contained <0.5% contamination by other blood cells. Total platelet membranes were prepared from platelet suspensions as previously described.3,10

Platelet membrane suspensions were labeled in the dark in the presence of 1 μM DPH in phosphate buffered saline for 60 minutes at 37°C.3,10 Labeled membranes contained approximately 1 probe molecule to 100 phospholipid molecules. Fluorescence was measured at 37.0°C (±0.1°C) with stirring on an SLM 4800 spectrofluorometer (Urbana, Ill.) equipped as previously described.3,10 Steady-state anisotropy measurements provide a reliable and valid index of membrane fluidity as reflected by a significantly lower mean corrected with age over 35 years,3,8,23 no age correction of the cutoff was employed.

Statistical analyses were performed using the SPSS® Version 3.0 software package.45 Univariate comparisons of continuous variables were made using a two-tailed t test. Univariate comparisons of discrete variables were made using the χ² statistic or Fisher's exact test, as appropriate. Logistic regression analysis was used to estimate the independent effects of risk factors for stroke in predicting the membrane fluidity subtype of demented patients and elderly controls. Life table analysis was used to explore the effect of membrane fluidity subtype on mortality rates, according to the method of Kaplan and Meier.46

Results

The demographic and clinical characteristics of the patient and control groups are compared in Table 2. The patients with Alzheimer's disease tended to be somewhat older and were significantly more cognitively impaired than the neurologically healthy elderly controls. The patient group also exhibited a significantly higher mean ischemic score than the controls, although both means were substantially below the cutoff value of 4 that has been used to differentiate primary degenerative dementia from dementia of vascular etiology.35 The patient and control groups did not significantly differ with respect to the average severity of cardiac disease or the dichotomous risk factors for stroke including the history of smoking, transient ischemic attacks, elevated hematocrit, or hypertension. Consistent with our previous reports, the patients with Alzheimer's disease exhibited increased platelet membrane fluidity as reflected by a significantly lower mean...
fluorescence anisotropy value of DPH-labeled membranes than the control group.

The same characteristics were examined within the patient group, after stratification according to platelet membrane fluidity (Table 3). Demented patients with increased platelet membrane fluidity were approximately 5 years younger on average than those with normal platelet membrane fluidity at the time of entry into our longitudinal study. Our previous reports suggest that this difference arises from an earlier age of onset of cognitive impairment in the former subgroup. Furthermore, the patients with increased membrane fluidity were significantly less likely to have a history of hypertension than the normal fluidity subgroup. A history of hypertension remained a significant independent predictor of membrane fluidity classification in a logistic regression analysis that simultaneously controlled for the effects of age and other risk factors for stroke (Table 4).

The results of this study were consistent with the hypothesis that hypertension, an important risk factor for cerebral ischemia, is less common among the subgroup of Alzheimer's disease patients with increased platelet membrane fluidity than among Alzheimer's disease patients who do not exhibit this membrane phenotype. Since vascular dementia is associated with a higher mortality rate than degenerative dementia, these results further suggested that Alzheimer's disease patients with increased platelet membrane fluidity might experience a decreased mortality rate compared with those who do not exhibit this membrane alteration. Only two (9.5%) of the 21 demented patients with increased membrane fluidity has died during longitudinal follow-up, while six (21.4%) of the 28 patients in the residual group (five patients were lost to follow-up) have died during the same interval ($p=0.24$, one-tailed Fisher's exact test). While the trend was in the predicted direction, the small sample size limited the statistical power of this comparison. Age-adjusted mortality rates obtained from life table analyses that yield age-corrected survival rates also revealed a similar trend toward a decreased rate of mortality for the Alzheimer's disease patients with increased platelet membrane fluidity, but this comparison was limited by a small sample size as well.

Univariate and logistic regression analyses were performed to compare the prevalence rates of risk factors for stroke between the subgroups of controls with increased or normal platelet membrane fluidity (Tables 5 and 6). In contrast to the results for the patient group, none of the risk factors for stroke discriminated between the membrane fluidity subgroups of neurologically healthy elderly controls. The univariate trend toward a difference in the prevalence of hypertension between the membrane fluidity

<table>
<thead>
<tr>
<th>Variable</th>
<th>Increased platelet membrane fluidity ($n=21$)</th>
<th>Normal platelet membrane fluidity ($n=28$)</th>
<th>Statistic</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>66.6±9.2</td>
<td>71.6±2.2</td>
<td>1.99</td>
<td>0.05</td>
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<tr>
<td>Ischemic score</td>
<td>2.4±2.0</td>
<td>2.6±2.2</td>
<td>0.39</td>
<td>0.70</td>
</tr>
<tr>
<td>Cardiac index</td>
<td>0.57±1.3</td>
<td>0.88±0.93</td>
<td>0.93</td>
<td>0.36</td>
</tr>
<tr>
<td>Dichotomous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>(7/14)</td>
<td>(6/22)</td>
<td>0.873</td>
<td>0.35</td>
</tr>
<tr>
<td>Smoking*</td>
<td>28.6% (6)</td>
<td>17.4% (4)</td>
<td>...</td>
<td>0.48</td>
</tr>
<tr>
<td>Transient ischemic attack</td>
<td>0.0% (0)</td>
<td>21.4% (6)</td>
<td>...</td>
<td>1.00</td>
</tr>
<tr>
<td>Carotid bruit</td>
<td>0.0% (0)</td>
<td>3.6% (1)</td>
<td>...</td>
<td>1.00</td>
</tr>
<tr>
<td>Hypertension</td>
<td>14.3% (3)</td>
<td>46.4% (13)</td>
<td>5.64</td>
<td>0.02</td>
</tr>
</tbody>
</table>

For continuous variables, values are mean±SD and statistic is $r$. For dichotomous variables, values are percentage of respective group expressing risk factor (number of cases in parentheses) and statistic is $\chi^2$. Probability determined by Fisher's exact test for smoking, transient ischemic attack, and carotid bruit. No case with abnormally elevated hematocrit was identified among patients with Alzheimer's disease.

* $n=23$ for normal fluidity.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.98</td>
<td>0.93-1.03</td>
</tr>
<tr>
<td>Ischemic score</td>
<td>1.38</td>
<td>0.97-1.97</td>
</tr>
<tr>
<td>Cardiac index</td>
<td>0.80</td>
<td>0.52-1.25</td>
</tr>
<tr>
<td>Sex</td>
<td>0.96</td>
<td>0.35-2.60</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.80</td>
<td>0.65-4.92</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.26*</td>
<td>0.07-0.90</td>
</tr>
</tbody>
</table>

| Platelet membrane fluidity subtype (increased=1, normal=0) was predicted in multiple logistic regression model from risk factors for stroke. Age, ischemic score, and cardiac index were coded as continuous variables. Age ranges for subgroups with increased and normal platelet membrane fluidity were 53–84 and 56–84 years, respectively. Remaining risk factors were dichotomous and were coded as 1 for conditions associated with increased risk of stroke and as 0 otherwise. Increased hematocrit was not included in this model since no cases of abnormally increased hematocrit were identified in pattern group. Furthermore, the few cases with transient ischemic attacks (six) or carotid bruit (one) precluded estimation of meaningful odds ratios associated with these variables. Odds ratios were significant when 95% confidence interval did not include unity.

*p<0.05.
subtypes of elderly controls (p=0.08, Table 5) was in the opposite direction from that observed for the patient group and did not approach statistical significance in the logistic regression model.

Discussion

In our study, Alzheimer’s disease patients with increased platelet membrane fluidity tended to be younger on average than Alzheimer’s disease patients with normal platelet membrane fluidity. However, platelet membrane fluidity is not correlated with age in the normal adult population, and this observation derives, instead, from the association of increased platelet membrane fluidity with an earlier symptomatic onset of dementia symptoms. In the current study, this membrane phenotype was also associated with a decreased prevalence of hypertension, an association that became even stronger when the effects of age and other risk factors for stroke were controlled for in a logistic regression analysis. In that model, the presence of hypertension decreased the likelihood that the patient belonged to the increased fluidity subgroup by a factor of nearly four (odds ratio=0.26). It should also be noted that increasing age and the presence of hypertension are the greatest risk factors for stroke among the risk factors that were included in our study. In addition, increased platelet membrane fluidity was associated with trends toward a lower ischemic score and cardiac disease index and a smaller fraction of cases who had a history of transient ischemic attacks or carotid bruits. Overall, these findings support the hypothesis that the clinical subgroup of Alzheimer’s disease patients with increased platelet membrane fluidity are at a lower risk for cerebrovascular disease than the subgroup of patients with normal platelet membrane fluidity. Moreover, these results suggest that focal EEG abnormalities in the context of clinically diagnosed Alzheimer’s disease may result from occult ischemic changes (not detected as an infarct by CT scan or neurological examination) in the brain.

Two potential explanations were considered for the association of increased platelet membrane fluidity with a decreased risk of cerebrovascular disease among patients with Alzheimer’s disease. Occult cerebral infarcts are not uncommonly found at autopsy in patients with histologically confirmed Alzheimer’s disease, and the prevalence of focal areas of ischemic change that appear to represent incomplete infarctions may be even more common. Therefore, the association of increased platelet membrane fluidity with decreased risk factors for stroke in Alzheimer’s disease patients could derive from the fact that this biological subgroup is more likely to suffer from a purely degenerative disorder, indirectly rendering the residual group more likely to include...
cases that have a vascular component to their dementias. Alternatively, the platelet membrane alteration may play a more direct role in the risk of stroke since platelets have been implicated in the formation of atheromatous plaques.²⁹,³⁰ It seems plausible, for example, that platelets with increased membrane fluidity (due to an abnormal internal membrane compartment) might be less able to aggregate or participate in the formation of atheromatous plaques and thereby confer a protective effect against cerebrovascular disease. A neurologically healthy control group, included in our study to distinguish between these alternative possibilities, did not exhibit an association of increased platelet membrane fluidity with decreased risk factors for stroke. Therefore, the association between increased platelet membrane fluidity and stroke risk appears to have resulted because the biological marker preferentially detected a more homogeneous subset of cases of primary neurodegeneration from a group of clinically diagnosed cases of Alzheimer’s disease with varying degrees of occult cerebrovascular disease and not from the fact that the membrane abnormality renders platelets less able to participate in the formation of atheromatous plaques.

In summary, Alzheimer's disease patients with increased platelet membrane fluidity exhibited a decreased prevalence of risk factors for stroke, as well as fewer cases with focal EEG changes,²⁰ than Alzheimer's disease patients with normal fluidity. The platelet membrane abnormality describes a clinical subgroup of patients with Alzheimer's disease who are less likely to have coexisting occult cerebrovascular disease than the remaining demented patients who meet NINCDS-ADRDA consensus clinical criteria for Alzheimer's disease.

Acknowledgments

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