Plasma Amyloid β-Protein and C-reactive Protein in Relation to the Rate of Progression of Alzheimer Disease

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Objective: To examine whether plasma markers of amyloid precursor protein metabolism (amyloid β-protein ending in Val-40 [Aβ40] and Ala-42 [Aβ42]), inflammation (high-sensitivity C-reactive protein), and folic acid metabolism (folic acid, vitamin B12, and total homocysteine levels) are associated with the rate of cognitive and functional decline in persons with Alzheimer disease.

Design: Longitudinal study across a mean (SD) of 4.2 (2.6) years with assessments at approximately 6- to 12-month intervals.

Setting: Outpatient care.

Patients: A cohort of 122 patients having a clinical diagnosis of probable Alzheimer disease, each with at least 2 assessments across time.

Main Outcome Measures: Scores on the cognitive Information-Memory-Concentration subscale of the Blessed Dementia Scale and the functional Weintraub Activities of Daily Living Scale.

Results: Low plasma levels of Aβ40, Aβ42, and high-sensitivity C-reactive protein were associated with a significantly more rapid cognitive decline, as indexed using the Blessed Dementia Scale, than were high levels. Low levels of Aβ42 and high-sensitivity C-reactive protein were significantly associated with more rapid functional decline on the Weintraub Activities of Daily Living Scale than were high levels. These plasma markers contributed about 5% to 12% of the variance accounted for on the Blessed Dementia Scale and the Activities of Daily Living Scale by fixed-effects predictors. Measures of folic acid metabolism were not associated with changes on either the Blessed Dementia Scale or the Activities of Daily Living Scale.

Conclusions: Plasma markers of amyloid precursor protein metabolism and C-reactive protein may be associated with the rate of cognitive and functional decline in patients with Alzheimer disease.

Arch Neurol. 2008;65(6):776-785

Alzheimer disease (AD) is a dementing illness with a mean duration of 8 to 10 years after the onset of memory impairment. The course of AD is relentlessly progressive, although the rate of progression of AD is highly variable; the standard deviation of the yearly decline in many continuous cognitive and functional measures is of the same magnitude as the mean rate of decline. For example, AD is associated with a mean (SD) worsening of 3 (4) points per year on the Mini-Mental Status Examination (MMSE); 3 (3) points per year on the Blessed Dementia Scale (BDS), Information-Memory-Concentration subscale (BDS); 9 (9) points per year on the cognitive subscale of the AD Assessment Scale; and 10% (10%) decline per year on the Weintraub AD-specific Activities of Daily Living (ADL) Scale. Clinical characteristics associated with more rapid decline in AD include early language impairment, psychiatric symptoms, and extrapyramidal features. Male sex, high educational achievement, early age at disease onset, and the apolipoprotein E (APOE) ε4 allele are inconsistently associated with more rapid rate of decline in AD. However, these demographic, clinical, and genetic factors explain only a small fraction of the variability in rates of progression. We hypothesized that biochemical measures of plasma proteins or metabolites implicated in AD risk can predict progression and account for some of the variability in rate of decline.

We used a candidate biomarker approach, selecting plasma measures implicated as risk factors for or correlates of the diagnosis of AD. This led us to investigate markers of inflammation (high-sensitivity C-reactive protein [hsCRP]),...
levels and the ratio of Aβ40 or Aβ42 were associated with the increased risk of development of AD in the Northern Manhattan Study of Aging and Dementia and in the Rotterdam Study, although recent studies suggest that plasma Aβ40 and Aβ42 levels and the ratio of Aβ40 to Aβ42 are lower in those at risk of dementia. An elevated tHcy level was associated with AD across 4 to 8 years in the Framingham Study, and an elevated hsCRP level was associated with cognitive impairment in several cross-sectional cohorts. While these and other plasma measures were studied in relation to disease risk and diagnosis, few have been evaluated as predictors of rate of progression of AD. Identification of biomarkers associated with progression of AD would provide insight into the pathophysiologic features of the disease, suggest targeted interventions in individuals with specific biochemical profiles, enable stratification by progression risk in clinical trials, and enable appropriate counseling and planning for patients and caregivers. Furthermore, some plasma biomarkers we studied can be modified by existing medications (eg, vitamin supplementation or statins) or compounds in early development (eg, γ-secretase inhibitors).

**METHODS**

**PATIENT DEMOGRAPHIC DATA AND MEASUREMENTS**

Plasma samples were collected in the Memory Disorders Unit at Massachusetts General Hospital from patients having a clinical diagnosis of probable AD. Informed consent for the study was obtained from the patients and caregivers by a staff physician (J.H.G. or M.C.I.) according to the provisions of the protocol approved by the Massachusetts General Hospital Institutional Review Board. Patients were enrolled from February 6, 2001, through June 11, 2002, during which blood samples were collected. They were followed up through November 11, 2005, for assessment of cognition (BDS) and function (Weintraub ADL Scale), with retrospective BDS and ADL data collected from as early as 1989 included in longitudinal analyses. In addition to BDS and ADL scores obtained concurrent with and after plasma collection, retrospective BDS and ADL data were used to increase power. This is justified by the stability of the subjects’ plasma values (see the “Results” section). All participants having a diagnosis of probable AD, available blood samples, and at least 2 clinic visits were included in the study. The following anonymized data were available for each subject: demographic data including sex, age, race/ethnicity, and date of birth, and educational achievement, and clinical characteristics including diagnosis, onset of disease based on the first symptoms of cognitive impairment reported by the patient’s spouse or other caregiver, and 2 measures of dementia severity obtained at each visit (BDS and ADL scores). The first BDS and ADL score for each subject was usually obtained at the first clinic visit and usually every 6 to 12 months thereafter. Subjects varied in terms of the number of visits analyzed and time in the study (Table 1). There was also variation in intervals between visits both within and between subjects (Table 1). However, our method of longitudinal data analysis (random-effects model) allows for such imbalanced data (see the “Statistical Analysis” subsection).

### Table 1. Statistical Data

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td></td>
</tr>
<tr>
<td>With BDS</td>
<td>122</td>
</tr>
<tr>
<td>With ADL</td>
<td>115</td>
</tr>
<tr>
<td>Total visits (% retrospective, ie, prior to blood drawing)</td>
<td></td>
</tr>
<tr>
<td>With BDS</td>
<td>919 (55)</td>
</tr>
<tr>
<td>With ADL</td>
<td>730 (38)</td>
</tr>
<tr>
<td>Age at baseline, mean (SD) [range], y</td>
<td>73.3 (8.2) [47-89]</td>
</tr>
<tr>
<td>Duration of Alzheimer disease at baseline, mean (SD) [range], y</td>
<td>3.2 (2.1) [0.7-12.4]</td>
</tr>
<tr>
<td>Educational achievement, mean (SD) [range], y</td>
<td>13.6 (3.1) [3-20]</td>
</tr>
<tr>
<td>Sex, No. (%)</td>
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</tr>
<tr>
<td>Males with BDS</td>
<td>55 (45)</td>
</tr>
<tr>
<td>Males with ADL</td>
<td>55 (45)</td>
</tr>
<tr>
<td>Females with BDS</td>
<td>67 (55)</td>
</tr>
<tr>
<td>Females with ADL</td>
<td>60 (52)</td>
</tr>
<tr>
<td>Age at onset of AD, mean (SD) [range], y</td>
<td>70.2 (8.4) [44-87]</td>
</tr>
<tr>
<td>No. of visits across time per person, mean (SD) [range]</td>
<td></td>
</tr>
<tr>
<td>BDS</td>
<td>7.5 (4.4) [2-26]</td>
</tr>
<tr>
<td>ADL</td>
<td>6.3 (3.2) [2-20]</td>
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<tr>
<td>Interval between visits, mean (SD) [range]</td>
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<tr>
<td>BDS</td>
<td>6 mo (4 mo) [2 wk-3 y]</td>
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<tr>
<td>ADL</td>
<td>9 mo (7 mo) [3 mo-6 y]</td>
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<td>ADL at baseline</td>
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<tr>
<td>ADL at blood drawing</td>
<td>43 (23) [6-87]</td>
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<td>Biochemical data, mean (SD) [range]</td>
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<td>Aβ40 level, pmol/L</td>
<td>51.5 (17.8) [12-110]</td>
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<td>Aβ42 level, pmol/L</td>
<td>7 (3.6) [2-22]</td>
</tr>
<tr>
<td>tHcy level, µmol/L</td>
<td>9 (3.2) [4-24]</td>
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<tr>
<td>Vitamin B12 level, pg/mL</td>
<td>600 (309) [187-1870]</td>
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<tr>
<td>Folic acid level, ng/mL</td>
<td>13.9 (9.9) [3-68]</td>
</tr>
<tr>
<td>hsCRP level, mg/L</td>
<td>2 (4.5) [0.1-3.46]</td>
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<td>Genetic data</td>
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<td>APOE genotype, No. (%)</td>
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</tr>
</tbody>
</table>

**Abbreviations:** Aβ40 and Aβ42, amyloid β-protein 40 and 42, respectively; ADL, Weintraub Activities of Daily Living Scale; APOE, apolipoprotein E; BDS, Blessed Dementia Scale; hsCRP, high-sensitivity C-reactive protein; tHcy, total homocysteine.

SI unit conversions: To convert vitamin B12 to picomoles per liter, multiply by 0.7378; to convert folic acid to nanomoles per liter, multiply by 2.266; to convert hsCRP to nanomoles per liter, multiply by 9.524.

*Except where noted, data pertain to the data set used in analysis of the BDS; values for the ADL data set were virtually the same, except where indicated.*

The BDS is a brief mental status test used to detect the presence and estimate the severity of cognitive impairments. It is administered by the examining physician and samples the domains of information, orientation, memory, and concentration. A BDS score of 0 to 3 mistakes is considered within the normal range, with a maximal score of 37 mistakes. The Weintraub ADL Scale is a 31-item questionnaire of basic and instrumental ADLs completed by the knowledgeable spouse or caregiver. The ADL score ranges from 0% (normal) to 100% (complete dependency). The biomarkers of interest were plasma levels of Aβ40 and Aβ42, amyloid β-protein ending in Val-40 [Aβ40] and Ala-42 [Aβ42], and homocysteine metabolism (total homocysteine [tHcy], folic acid, and vitamin B12 levels). High plasma levels of Aβ40 or Aβ42 were associated with the increased risk of development of AD in the Northern Manhattan Study of Aging and Dementia and in the Rotterdam Study, although recent studies suggest that plasma Aβ40 and Aβ42 levels and the ratio of Aβ40 to Aβ42 are lower in those at risk of dementia. An elevated tHcy level was associated with AD across 4 to 8 years in the Framingham Study, and an elevated hsCRP level was associated with cognitive impairment in several cross-sectional cohorts. While these and other plasma measures were studied in relation to disease risk and diagnosis, few have been evaluated as predictors of rate of progression of AD. Identification of biomarkers associated with progression of AD would provide insight into the pathophysiologic features of the disease, suggest targeted interventions in individuals with specific biochemical profiles, enable stratification by progression risk in clinical trials, and enable appropriate counseling and planning for patients and caregivers. Furthermore, some plasma biomarkers we studied can be modified by existing medications (eg, vitamin supplementation or statins) or compounds in early development (eg, γ-secretase inhibitors).
and Aβ42, the ratio of Aβ42 to Aβ40, and hsCRP, tHcy, folic acid, and vitamin B12 levels measured during the course of follow-up in the clinic without regard to the subject’s stage of illness.

There were 122 subjects who had at least 2 clinic visits with BDS scores recorded for longitudinal analyses (Table 1 and Figure 1). There were 115 subjects who had at least 2 visits with an ADL score (1 of these subjects was not among those included in the BDS data set). Almost 80% of the patients in this study were taking a cholinesterase inhibitor at the time of blood collection; none were taking memantine. To ensure that medication use did not affect the results of the study, we compared all biomarker measures between subjects who were taking cholinesterase inhibitors and those who were not. There were no significant differences for any marker between these groups, making it unlikely that medication use might have influenced the results of the study.

**BLOOD COLLECTION**

A trained phlebotomist collected 22.5 mL of blood from each subject in three 7.5-mL polypropylene sterile plunger tubes (S-Monovette blood collection system; Sarstedt, Inc, Newton, North Carolina) containing potassium EDTA. The blood samples were cooled to 4°C for 15 minutes. A serum-plasma separator was added (Sure-Sep II; Organon USA, Inc, West Orange, New Jersey). In rapid succession, the samples were centrifuged at 3300 rpm (1380g) for 15 minutes; 960-µL aliquots of plasma were placed in polypropylene tubes containing protease inhibitor cocktail, 40 μL/ Complete 25X; Roche Diagnostics, Basel, Switzerland) in phosphate-buffered saline solution, then frozen on dry ice. The samples were stored at −80°C until ready for use. The protease inhibitor cocktail did not interfere with any of the assays.

**BIOCHEMICAL ASSAYS**

Plasma Aβ40 and Aβ42 concentrations were determined using a sandwich enzyme-linked immunosorbent assay using the BNT77 capture antibody and C-terminal–specific detector antibodies BA27 and BC05 as described. The plasma tHcy level was determined using high-performance liquid chromatography with fluorometric detection; the plasma hsCRP level was determined using nephelometric detection; and plasma folic acid and vitamin B12 levels were determined using a radioimmunoassay (BioRad Quantaphase II kit; BioRad Laboratories Inc, Hercules, California).

**STATISTICAL ANALYSIS**

The outcomes of interest were the BDS and ADL scores. Patients with cognitive impairment so severe at a given session that they could not complete the BDS were rated as untestable at that session. To increase power in these cases, we used an algorithm to estimate missing BDS and ADL scores that assigned a score of 32 (99.5th percentile) on the BDS and 77% dependency on the ADL (median ADL score when the BDS was rated as untestable). Only the first so estimated BDS or ADL per person was used, to avert an artificial plateau effect. This allowed 1 ceiling value to be incorporated in the analysis for patients with cognitive impairment so severe as to be untestable. Of records available for longitudinal analysis, 4% of BDS and 2% of ADL scores were so estimated. Each subject had to have at least 2 nonmissing BDS and ADL scores, including estimated scores, to qualify for the respective longitudinal analyses.

Only 1 value for each of the plasma measures per subject (the earliest) was used in the longitudinal analyses. A subset of subjects with repeated measures obtained about 1 year apart was used to examine the stability and reliability of these measures over time in a separate analysis (see “Results” section).

A mixed fixed- and random-coefficient longitudinal regression analysis was conducted to assess the relationship of a given plasma measure to level and change in mean BDS or ADL scores across time, considering the correlation between repeated outcome measures in the same individual. A separate analysis was conducted for each plasma variable and for the BDS and the ADL as outcomes, respectively.

For each plasma variable, we constructed an optimal mixed fixed- and random-effects regression model for the BDS (or ADL) scores of the patients in the study. All available scores, before and after plasma collection, were used. The regression models included a random intercept, and random linear and quadratic (single-bend curvilinear) terms for duration of AD illness at a visit (years since the patient or caregiver first noticed memory problems). Disease duration obtained from informant interview, while imprecise for a disease with insidious onset such as AD, is a robust metric of progression and dementia severity, correlating strongly with BDS and neuropathologic findings at death. Linear and quadratic terms for years as a clinic patient at the time of the visit were additionally included as fixed predictors to adjust for possible effects of clinical care on progression. Preliminary graphic analysis of raw data suggested some nonlinearity (Figure 1); therefore, quadratic terms were included to model accelerating or decelerating progression and to permit adequate fit of ceiling and floor asymptotes (random quadratic terms could fit both in the same model, if necessary, which was the case for the ADL). An additional benefit of explicitly modeling curvilinearity is that there is no need to covary a baseline term because an otherwise computed linear rate would depend on baseline if the true underlying progression is curvilinear. Other fixed covariates assessed were years of educational achievement, and linear and quadratic components of patient age at the time of the visit.

We conducted secondary sensitivity analyses in which slightly different longitudinal models were used to determine the robustness of any effects we found. For example, we modeled duration at baseline and age at baseline as constant covariates across time with years in the study as a random time-varying predictor. We also removed years as a patient in the memory clinic entirely, including only duration (random effect) and age at time-varying predictors. To test whether any of the biomarkers influenced the level or trajectory of BDS and ADL scores over time, each plasma variable was entered as a separate fixed term in the model along with its interactions with the linear and the quadratic components of duration of disease at the visit and years as a clinic patient. The saturated model containing all terms was subjected to backward elimination at $P \leq .05$ for retention of terms (nonsignificant lower-order correspondents to significant higher-order terms were permitted). Significance and retention of the interaction terms of the plasma variables with duration of illness or time as a clinic patient indicate that the biomarker influenced the trajectory of BDS or ADL scores over time. Retention of only the main effect for the plasma measure indicated that it was associated with overall level of BDS (ADL) across time, that is, with the mean BDS (ADL) score across all of a subject’s visits, though it was not predictive of differential change across time.

Unstructured correlations among random intercept, linear, and quadratic terms were estimated. The model was estimated with restricted maximum likelihood methods using commercially available software (SAS version 9.1.3, the Mixed Procedure; SAS Institute Inc, Cary, North Carolina). The distributions of the plasma measurements and other predictors in the models are not required to be normally distributed; residuals are (see the “Results” section). However, the hsCRP measure was so positively skewed that we log-transformed it to normality to
Figure 1. Raw scores on the Blessed Dementia Scale (BDS) (A) and the Weintraub Activities of Daily Living Scale (ADL) (B) across the duration of Alzheimer disease in years. Thin lines connect scores for the same person. Thick lines indicate ordinary least squares linear regression line (solid red lines) and quadratic curve (dashed blue lines). Unlike the random coefficient analyses, the ordinary least squares lines are blind to the within-subject vs between-subject distinction and the correlation of scores across time within a subject. The ordinary least squares lines tend to underestimate the within-subject rates, which is noticeable in the figure.
In random intercept models, we found that only 1 plasma or absence of the classification variable for presence or absence of the ing approaches were the same as those described except that a longitudinal analyses. The random- and fixed-effects modeling fixed terms were not retained. statistically significant (variances always were) even if correspond-

covariances of random terms were retained in the model if sta-

Avoid excessive influence of extreme values. The variances and covariances of random terms were retained in the model if statistically significant (variances always were) even if corresponding fixed terms were not retained.

As an ancillary issue, we analyzed the relation of presence or absence of the APOE ε4 allele to the BDS and ADL scores in longitudinal analyses. The random- and fixed-effects modeling approaches were the same as those described except that a classification variable for presence or absence of the APOE ε4 allele and its interactions with duration of illness and years as a clinic patient were included in place of analogous terms for a plasma measure variable.

RESULTS

STABILITY AND RELIABILITY OF PLASMA MEASURES

None of the plasma measurements correlated strongly with patient age or duration of AD at blood collection, except for a moderate positive correlation between age and tHcy level (r = 0.36, P = .001). In assessing how a single reading of a given plasma measure obtained at a point during the course of AD is related to change in dementia symptom measurements over time, the stability of the plasma measure itself across time within a person needs to be considered. We had some limited data to address this issue. For 49 patients with neurologic disorders (28 patients with AD [57%], all but one of whom were included in the longitudinal analyses; 11 with Parkinson disease [22%]; 6 with mild cognitive impairment [12%]; and 4 miscellaneous [8%]), we had 2 measures of Aβ40 and of Aβ42 obtained a mean (SD) of 1 (0.1) year apart. For 10 patients, we had 2 measures of tHcy, hsCRP, fol-

cic acid, and vitamin B12 levels obtained 1 (0.2) year apart. In random intercept models, we found that only 1 plasma measure, Aβ42, showed a significant (P < .001), though small, mean change across time (mean decline of 1.5 pmol/L/year). Furthermore, a fixed-effect general linear model with subjects as a classification variable indicated that subjects accounted for 64% to 93% of the variance for a given plasma measure (all significant, P ≤ .01). These are essentially intraclass correlation coefficients. (The remaining percent variance is “error” in this context, owing to the effect of time and the interaction of subjects with time.) Thus, the plasma measures showed fairly good stability across the duration of the study except possibly for Aβ42, which showed a slight tendency to decline with time.

LONGITUDINAL ANALYSIS OF EFFECT OF PLASMA MEASURES AND APOE ε4 GENOTYPE ON COGNITIVE DECLINE: BDS

None of the plasma measures, including the ratio of Aβ42 to Aβ40, correlated with baseline BDS scores or BDS scores on the date of blood collection, which further suggests that there was no need to covary for a baseline con-

found, and we were, thus, able to use the baseline to aug-

ment the time-related dependent variable. The plasma measures also did not correlate with time in the study or number of visits, which suggests that plasma levels were not confounded with any differential attrition. In the lon-

itudinal analyses, no plasma measure was found to have an association solely with the mean level of BDS across the entire duration of the study.

The only plasma measures showing significant relations to change in BDS score over time were Aβ40, Aβ42, and log-

hsCRP. In each case, higher values were associated with slower rate of deterioration in cognitive functioning (Figure 2).

The ratio of Aβ42 to Aβ40, and the levels of folic acid, tHcy, and vitamin B12 did not influence rate of BDS progression. For Aβ40, there was a significant (P = .04) linear effect of age in increasing (worsening) BDS scores as well as quadratic effects of duration of AD and years as a clinic patient. The Aβ40 level significantly (P < .02) interacted with the quadratic term for duration and with the linear term for years as a clinic patient (P < .001), indicating that the level of plasma Aβ40 influenced the trajectory of the BDS score over time. These effects are shown in Figure 2A not only in the different rate of change associated with different levels of Aβ40 but also in the different degree of curvature. Results for the analysis of the level of Aβ42 were similar to those for the level of Aβ40 except that duration of illness had a significant (P = .05) linear (worsening) effect only on the BDS score and the level of Aβ42 significantly (P = .001) interacted only with the linear term for years as a clinic patient (Figure 2B).

In the case of log-hsCRP, age was not significantly related to the BDS score, although duration of illness and years in the study had quadratic relations and log-hsCRP significantly (P = .04) interacted with the quadratic component of AD du-

ration, resulting in the effects shown in Figure 2C. Residu-

als from these models were fairly normally distributed in con-

formance with assumptions and consistent with good fit.

Sensitivity analyses of alternate models for the time variables (see the “Statistical Analysis” section) yielded similar results; effects of major interest were in the same direction and their significance levels on the same order as before, with predicted curves almost the same. As an additional check of the robustness of our findings, we reran all analyses with significant marker effects, this time including only subjects with 3 or more visits. Although persons with only 2 visits contribute partial and between-subject information to all relations (especially for the time invariant and linear predictors), requiring at least 3 ob-

servations per person enables assessment of within-subject quadratic relations to be more intuitively meaningful. Fewer than 12 subjects (10%) and 24 records (3%) were removed. Coefficient estimates, P values, and predicted curves were found to be nearly the same as before. The biomarker terms of most interest remained statistically significant, and sometimes more so.

Because the significant time-related effects involved complicated curvilinear terms and interactions, numerical coefficient estimates do not provide an intuitive sense of how the plasma measures affect rate of change in the BDS score. However, inspection of Figure 2 shows that relations are close enough to linear to permit estimates of how increase in the plasma measure is related to change in progression in the BDS score for reasonable time ranges. Table 2 gives these estimates of rate and rate change for selected percentiles of biomarkers involved in significant effects for the BDS.

Another perspective on effect size for rate of decline can be obtained by determining the percent variance ac-
Figure 2. Illustrative mean scores on the Blessed Dementia Scale (BDS) across time predicted by the fitted model in the longitudinal analysis for selected levels of amyloid β-protein 40 (A), amyloid β-protein 42 (B), and log-transformed, high-sensitivity C-reactive protein (C) and an example time span (maximum likelihood estimates). For this graph, age was set at 75 years and years as a patient in the memory clinic at zero when duration of Alzheimer disease was 3 years, in correspondence to the approximate relation of their respective actual mean values at first visit in the data. Age, years as a clinic patient, and duration of illness were then increased in tandem. Illustrative levels of the biomarker were chosen to correspond to the 1st, 25th, 50th (median), 75th, and 99th percentiles of its distribution. Thus, the spacing between the lines reflects the shape of the distribution. The vertical dotted line indicates the point at which the patient was first seen in the memory clinic, that is, where years as a patient in the memory clinic was zero in the model.
counted for in the BDS score by a given plasma measure across the longitudinal design. For the analysis of Aβ40 and Aβ42 levels, about 19% of the variance in the BDS scores was accounted for by the fixed terms from the optimal derived model (including the fixed portions of duration of illness) without including the terms involving Aβ (and not including all the variance owing to subject differences; ie, a simple fixed model was run). The figure increased by about 2.2% to 2.3% when the Aβ terms were included, or about 10% to 12% of the variance accounted for by all fixed terms. This is not an insubstantial amount relative to pooled effects of all of the significant predictors, that is, age, duration of illness, and years as a clinic patient (linear and quadratic effects of each). Log-hsCRP accounted for about 7.7% of the variance accounted for by all of the fixed terms.

Insofar as the analysis for effects associated with APOE, preliminary tests showed no significant differences in demographic data (sex, age at blood drawing, age at onset of AD, duration of AD, and educational achievement) or levels of plasma markers between the groups with and without an APOE ε4 allele, except that the group without the allele had a significantly higher level of hsCRP (P = .06 by permutation test; P = .01 for log-hsCRP). The APOE ε4 allele did not affect the rate of cognitive decline, although it was associated with a marginally higher overall level of impairment across the duration of the study (P = .08), which may have been connected to its association with lower hsCRP levels.

LONGITUDINAL ANALYSIS OF EFFECT OF PLASMA MEASURES AND APOE ε4 GENOTYPE ON FUNCTIONAL DECLINE: ADL

None of the plasma measures, including the ratio of Aβ42 to Aβ40, correlated with the ADL score at baseline or on the date of blood collection or with time in the study or number of visits. In the ADL longitudinal analyses, all plasma measures, as well as the ratio of Aβ42 to Aβ40, were associated with significantly different trajectories of change over time, except for Aβ40 and vitamin B₁₂ levels, which did not have significant associations with either level or change in the ADL score. However, effect sizes were small in all cases except for the analysis for the level of Aβ42 and log-hsCRP, where there were significant quadratic effects for age (P < .001) and for duration of illness, with the plasma measure interacting with the quadratic term for duration (P < .05). Similar to the BDS results, higher levels of Aβ42 and log-hsCRP were associated with slower deterioration on the ADL Scale (Figure 3). In both the Aβ42 and log-hsCRP analyses, years as a clinic patient also had a significant linear worsening effect (P < .001), and higher educational achievement significantly predicted a lower level of impairment across the study (P < .02). Residuals from these models were fairly normally distributed in conformance with assumptions and consistent with good fit. Sensitivity analyses showed little change in results of primary interest. Further, restricting analyses to only subjects with 3 or more visits (7 subjects [6%] and 14 observations [2%] were removed) showed negligible changes in significant effects of primary interest.

As computed for the BDS, we estimated decreases in rate of change associated with higher levels of the plasma measures (Table 2). The terms in the model for the level of Aβ42 contributed 7.5% of the variance in the ADL scores accounted for by all fixed terms. Log-hsCRP contributed approximately 5% of the fixed-effect variance accounted for. The APOE ε4 allele did not affect the ADL rate of deterioration or correlate with overall level of functional dependency.

<table>
<thead>
<tr>
<th>Percentile of Biomarker</th>
<th>Aβ40</th>
<th>Aβ42</th>
<th>Log-hsCRP</th>
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<td>5.0</td>
<td>5.4</td>
<td>11.0</td>
<td>12.0</td>
</tr>
<tr>
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<td>4.6</td>
<td>4.4</td>
<td>4.6</td>
<td>11.0</td>
<td>10.6</td>
</tr>
<tr>
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<td>9.4</td>
<td>9.2</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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<td>3.0</td>
<td>7.0</td>
<td>4.0</td>
</tr>
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</table>

Abbreviations: Aβ40 and Aβ42, amyloid β-protein 40 and 42, respectively; ADL, Weintraub Activities of Daily Living Scale; BDS, Blessed Dementia Scale; hsCRP, high-sensitivity C-reactive protein.

To our knowledge, this study is the first to examine the effects of putative biochemical risk factors and biomarkers for dementia on the course of illness in patients with AD, and we found that differences in plasma markers of amyloid precursor protein metabolism and CRP explain a statistically significant and clinically meaningful percentage of the variability in clinical progression of AD. We used scores from a mental status examination (BDS) and the ADL Scale to index and track the progression of dementia for more than 4 years. Scores for both measures worsened with time. The ADL Scale reached a ceiling effect much later in the course of illness compared with the BDS. This observation suggests that scales capturing instrumental and basic ADLs may have better operating characteristics than cognitive scales in long-term studies for follow-up of patients with advanced disease. Among the plasma biomarkers we examined, Aβ40, Aβ42, and hsCRP levels affected the rate of cognitive decline as judged with the BDS. The level of Aβ42 also affected the rate of functional impairment detected on the ADL Scale, as did the level of hsCRP. Low levels of plasma Aβ40, Aβ42, and hsCRP were associated with more rapid progression of illness. In the
Figure 3. Illustrative mean scores on the Weintraub Activities of Daily Living Scale (ADL) across time predicted by the fitted model in the longitudinal analysis for selected levels of amyloid β-protein 42 (A) and the log-transformed, high-sensitivity C-reactive protein (B) and time span (maximum likelihood estimates). Age at first visit was set at 75 years and years as a patient in the memory clinic at zero. Years of educational achievement was also significantly related to better scores and was set at the grand mean (14 years) for these predicted values. Illustrative levels of the biomarker were chosen to correspond to the 1st, 25th, 50th (median), 75th, and 99th percentiles of its distribution. The vertical line indicates the point at which the patient was first seen in the memory clinic, that is, where years as a patient in the memory clinic was zero in the model.
ADL analysis, the ratio of Aβ42 to Aβ40, and tHcy and folic acid levels also significantly influenced the rate of functional decline, but effect sizes were small and probably of little clinical significance. We attribute detection of these minor effects to high statistical power owing to the large number of subjects in this study and numerous observations per subject (730 ADL records).

Cross-sectional studies of plasma Aβ report high, unchanged, or low levels in AD or mild cognitive impairment relative to control subjects. In cross-sectional studies, plasma Aβ levels were not associated with severity of dementia. The Northern Manhattan Study found that high baseline plasma Aβ levels were associated with increased risk of AD over 3 to 5 years and that Aβ42 levels declined more rapidly in individuals developing AD. In the Rotterdam Study, high Aβ40 was associated with increased risk of dementia over 8 or 9 years. Both of the latter longitudinal studies reported that a more rapid decline in plasma Aβ42 levels occurred with incident dementia. Recent studies find that low Aβ40 or Aβ42 levels or the ratio of Aβ42 to Aβ40 are risk factors for mild cognitive impairment and AD. A low Aβ42 level is also associated with depression in elderly persons, a common comorbidity of AD. Our study extends these results to the effects of Aβ40 and Aβ42 in established AD: low rather than high levels of plasma Aβ were associated with more rapid cognitive and functional decline in AD. We speculate that low plasma Aβ levels reflect sequestration of Aβ in the brain, similar to the mechanism proposed for low Aβ42 levels in the cerebrospinal fluid. Before Aβ deposition in the brain, a high plasma Aβ level may reflect genetic predisposition to increased production or reduced clearance of Aβ that may lead to AD; with the initiation of Aβ deposition in the brain, plasma Aβ levels seem to decline; once the disease is established, the lowest Aβ levels seem to predict more rapid progression. However, this does not explain how plasma Aβ levels can influence AD risk and AD progression, yet not be a reliable diagnostic marker in cross-sectional studies including those with pathologic confirmation.

In our cohort, high hsCRP levels were associated with slower decline. Published studies tended to show the opposite; that is, high hsCRP levels were associated with cognitive decline. While this result may reflect true pathophysiologic effects on AD progression, we caution that this effect was less significant than the Aβ effects and may be driven by the large sample size and outliers. Furthermore, unlike plasma Aβ, which tends to be resistant to the effects of existing medication, the effects of hsCRP can be confounded by medications (eg, nonsteroidal anti-inflammatory drugs and statins), which we did not consider in our analyses.

Approaches to modeling progression in AD include survival analysis using landmark clinical stages, summary measures analysis, latent growth curve analysis, 2-stage regression, generalized estimating equations, and random-effects models. We emphasized the random-effects model in our analysis because it has advantages in assessing rate of progression of AD. The analysis explicitly models individual trajectories. Additional covariates are incorporated as fixed or random effects, and the biomarker by time (and time squared) interactions allow the mean trajectory of decline to differ by biomarker level. The model accommodates unbalanced and incomplete data, with differences in the number of observations for each subject and different times and spacing of observations within and between subjects, allowing full use of the observed data. The model accounts for correlation among repeated measures; the correlation is modeled as a continuous function of time, requiring fewer parameters to estimate the covariance between repeated measures in the same individual.

Random-effects models were used to assess factors related to rate of cognitive decline in several studies. A community-based Korean study of progression in 107 patients with AD found that the rate of decline during 1 year, based on a screening test (MMSE), cognitive subscale of the AD Assessment Scale, and ADL Scale (Disability Assessment for Dementia), was not associated with the severity of dementia, sex, educational achievement, duration of AD, or associated health problems. Age was associated with more rapid decline in MMSE but not on the other measures. High educational achievement and left-handedness were associated with more rapid decline on the MMSE in a patient population similar to ours, according to a study from the Johns Hopkins Alzheimer's Disease Research Center. In other studies, random-effects models were used to characterize the extent of placebo effect in AD treatment trials.

Random-effects models were used to assess rate of progression relative to APOE ε4 on progression have been inconsistent across studies. For example, in one report, the presence of an APOE ε4 allele was associated with more rapid decline in the cognitive portion of the Cambridge Examination for Mental Disorders of the Elderly. In another, the APOE ε4 allele was also associated with more rapid decline in MMSE score, but the relationship disappeared after multivariate adjustment. In the present study, we did not detect an effect of the APOE ε4 allele on rate of progression; this finding is similar to our previous conclusion based on neuropsychological testing outcomes.

Our results should be interpreted in the context of the potential limitations of this study. False-negative results can arise from lack of power or regression dilution, but these theoretical concerns were not an issue in our study. The study was adequately powered to detect continuous effects of these markers; indeed, the ADL analysis was powerful enough to detect effects that were statistically but not necessarily clinically significant. In a subset of our subjects, we also investigated whether a single measurement reflected long-term biomarker stability. Over 1 year, aside from a slight decline in the level of Aβ42, there was little overall change in these measures, which suggests little effect of regression dilution on these results. Nevertheless, the study does not address whether longitudinal changes in the markers themselves are correlated with the rate of progression. We cannot exclude the possibility that medications could have affected plasma tHcy, folic acid, hsCRP, or vitamin B12 levels. Almost 96 patients (80%) in this study were taking a cholinesterase inhibitor at the time of blood drawing, and more were taking a cholinesterase inhibitor during follow-up; thus, we could not evaluate independent effects of cholinesterase inhibitors on progression. This
This study was supported by grants from the National Institutes of Health, the American Federation for Aging Research (Dr Growdon), and T32NS048005 (Dr Irizarry). AG05134 (Massachusetts Alzheimer’s Disease Research Center) (Dr Growdon). Financial Disclosure: None reported. Funding/Support: This study was supported by grants AG05134 (Massachusetts Alzheimer’s Disease Research Center) (Dr Growdon) and T32NS048005 (Dr Irizarry) from the National Institutes of Health, the American Federation for Aging Research (Beeson Award) (Dr Irizarry), the J. D. French Alzheimer’s Foundation (Dr Irizarry), and the Lawrence J. and Anne Cable Rubenstein Foundation (Dr Growdon). Additional Contributions: David Schoenfeld, PhD, Harvard Medical School, Boston, provided valuable consultation on statistical issues.

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