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Amyloid-Beta (1-40) and the Risk of Death From Cardiovascular Causes in Patients With Coronary Heart Disease

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ABSTRACT

BACKGROUND The amyloid beta peptide is the major protein constituent of neuritic plaques in Alzheimer disease and appears to play a central role in vascular inflammation pathophysiology.

OBJECTIVES This study sought to determine the clinical value of amyloid-beta 1-40 (Abeta40) measurement in predicting cardiovascular (CV) mortality in patients with coronary heart disease (CHD) and arterial stiffness progression in young healthy subjects.

METHODS Abeta40 was retrospectively measured in blood samples collected from 3 independent prospective cohorts and 2 case-control cohorts (total N = 1,464). Major adverse cardiac events (MACE) were assessed in the 2 prospective cohorts (n = 877) followed for a median of 4.4 years. To look at effects on subclinical disease, arterial stiffness was evaluated at baseline and after 5-year follow-up (n = 107) in young healthy subjects. The primary endpoint was the predictive value of Abeta40 for CV mortality and outcomes in patients with CHD.

RESULTS In Cox proportional hazards models adjusted for age, sex, estimated glomerular filtration rate, left ventricular ejection fraction, high-sensitivity C-reactive protein, and high-sensitivity troponin T, Abeta40 independently predicted CV death and MACE in patients with CHD ($p < 0.05$ for all). After multivariate adjustment, Abeta40 levels conferred a substantial enhancement of net reclassification index and integrated discrimination improvement of individuals at risk in the total combined CHD cohort over the best predictive model. Further cohort-based analysis revealed that Abeta40 levels were significantly and independently associated with arterial stiffness progression, incident subclinical atherosclerosis, and incident CHD.

CONCLUSIONS Measuring blood levels of Abeta40 identified patients at high risk for CV death. (J Am Coll Cardiol 2015;65:904–16) © 2015 by the American College of Cardiology Foundation.

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Prevention and risk management of atherosclerotic vascular disease complications, which are the main causes of mortality and morbidity, remain major health challenges, with a tremendous socioeconomic impact on Western societies. Identifying the clinical value of novel biological pathways involved in coronary heart disease (CHD) may not only reveal new therapeutic targets but also improve risk stratification in secondary prevention. The high rate of cardiovascular (CV) death and the substantial heterogeneity in risk profiles among CHD groups underline the importance of a personalized approach to secondary prevention (1). Identifying blood-based biomarkers offering independent prognostic value and significant reclassification ability could be tremendously helpful in risk stratification and appropriate management.

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Cumulative evidence suggests that diseases caused by age-related chronic sterile inflammation, such as atherosclerosis and Alzheimer disease, may not only share genetic and environmental risk factors but also have common molecular mechanisms (2). Amyloid-beta 1-40 (Abeta40) peptides are generated by proteolytic cleavage of the amyloid precursor protein (APP) by beta- and gamma-secretases (3). Increasing evidence from mouse models points toward a central role for APP and Abeta40 in vascular inflammation pathophysiology (4–8). Abeta40 activates a cascade of proinflammatory events in endothelial cells and macrophages involving cytokine secretion and oxidative stress leading to vascular disease (6,9,10). Although Abeta40 is the predominant amyloid-beta peptide found in human atherosclerotic lesions (11), its clinical value has not been investigated in patients with CHD. We hypothesized that the proinflammatory potential of Abeta40 peptide may be associated with the clinical manifestation of atherosclerosis in humans and with major adverse cardiac events (MACE).

This study's primary aim was to evaluate the predictive value of Abeta40 blood levels in 2 independent cohorts of patients with stable CHD and assess the association of Abeta40 levels with incident CHD as well as the extent and progression of subclinical atherosclerosis.

METHODS

The present study involved 3 prospective and 2 case-control cohorts comprising 1,464 patients in hemodynamically stable condition who had been recruited in 3 European CV centers. First, patients undergoing diagnostic coronary angiography at the German Heart Centre in Munich (n = 514) were consecutively recruited, as previously described (12). The prospective part of the Munich cohort consisted of 321 consecutive patients with angiographically confirmed stable CHD who were followed for a median of 49.1 months for MACE. Patients with a normal coronary angiogram recruited during the same time period were used as controls (n = 193). The Athens cohort consisted of 556 consecutive patients age <75 years with creatinine levels of <2 mg/dl and stable CHD followed for a median of 61 months for MACE, as previously described (13). The third prospective cohort consisted of young healthy subjects without clinically overt CHD who has arterial stiffness and Abeta40 levels evaluated at baseline and after a 5-year follow-up period (n = 107). Finally, the peripheral atherosclerosis assessment cohort (n = 394) was an ambulatory case-control cohort including patients without clinically overt CHD (control subjects; n = 272) and patients with angiographically verified stable CHD (cases; n = 122). Patients with acute coronary syndrome (ACS), acute or chronic inflammatory conditions, infection, cancer, or stroke were not included in any of the cohorts. The local ethics committee of each university center approved the individual cohort studies, and patients provided written informed consent.

Blood samples for Abeta40 measurement were obtained as described in the Online Appendix. Plasma concentrations of high-sensitivity C-reactive protein (hsCRP) and high-sensitivity troponin T (hsTnT) were measured as previously described (12). All laboratory measurements were performed by experienced staff blinded to clinical characteristics. Peripheral atherosclerosis was assessed (14), and a detailed description of the assessment is available in the Online Appendix.

STATISTICAL ANALYSIS. We used multivariable Cox proportional hazard models to examine the association

ABBREVIATIONS AND ACRONYMS

Abeta40 = amyloid-beta 1-40
APP = amyloid precursor protein
CHD = coronary heart disease
CV = cardiovascular
eGFR = estimated glomerular filtration rate
hsCRP = high-sensitivity C-reactive protein
hsTnT = high-sensitivity troponin T
IMT = intima media thickness
LVEF = left ventricular ejection fraction
MACE = major adverse cardiac event(s)
MI = myocardial infarction
PWV = pulse-wave velocity

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TABLE 1 Characteristics of CHD Cohorts

	Overall	1st Quartile	2nd Quartile	3rd Quartile	4th Quartile	p Value*
Munich cohort (subcohort with angiographically confirmed CHD; n = 321)						
Participants, n	321	83	78	80	80	
Mean plasma Abeta40, pg/ml	114.9 (29.8)	84.1 (13.1)	104.7 (3.5)	117.9 (4.7)	153.7 (27.4)	<0.001
Mean age, yrs	67.2 (9.9)	62.5 (10.4)	64.6 (8.6)	68.5 (8.8)	73.3 (8.3)	<0.001
Female	75 (23.4)	14 (16.9)	8 (10.3)	29 (36.3)	24 (30)	<0.001
CV risk factors						
Hypertension	22 (69.2)	50 (60.2)	55 (70.5)	61 (76.3)	56 (70)	0.166
Diabetes mellitus	99 (30.8)	25 (30.1)	22 (28.2)	27 (33.8)	25 (31.3)	0.897
Smoking	34 (10.6)	15 (18.1)	7 (9)	9 (11.3)	3 (3.8)	0.028
Family history of CHD	138 (43)	36 (43.4)	36 (46.2)	37 (46.3)	29 (36.3)	0.272
Clinical parameters						
SBP, mm Hg	151 (28.4)	148 (27.4)	148 (23.2)	160 (26.8)	152 (34.1)	0.051
Cholesterol, mg/dl	181 (42.5)	183 (41.2)	184 (49.1)	184 (40.7)	173 (38.7)	0.302
HDL cholesterol, mg/dl	52.2 (14.5)	52.9 (14.8)	52.5 (13.3)	53.5 (16.2)	50 (13.5)	0.472
LDL cholesterol, mg/dl	100 (37.2)	104 (39)	103 (40.1)	99.2 (33.9)	95.2 (35.6)	0.452
Median CRP, mg/l (IQR)	1.69 (3.12)	1.56 (2.80)	1.68 (2.73)	1.61 (2.34)	2.51 (5.34)	0.058
Median eGFR, ml/min/1.73 m^2 (IQR)	88 (42.1)	100 (38.8)	93.4 (27.9)	85.3 (37.4)	59.6 (33.8)	<0.001
LVEF, %	56.3 (9.4)	56.3 (10.2)	57 (8.9)	57.6 (8.6)	54.3 (9.7)	0.129
CV endpoints						
CV death	22 (6.9)	4 (4.8)	1 (1.3)	3 (3.8)	14 (17.5)	<0.001
CV death and AMI	28 (8.8)	7 (8.4)	2 (2.6)	3 (3.8)	16 (20.0)	<0.001
Extended composite endpoint	59 (18.4)	14 (16.9)	8 (10.3)	8 (10.1)	29 (36.2)	<0.001
CV drugs						
Antiplatelets (aspirin/clopidogrel)	320 (99.7)	82 (98.8)	78 (100)	80 (100)	80 (100)	0.668
Beta-blockers	314 (97.8)	80 (96.4)	77 (98.7)	78 (97.5)	79 (98.8)	0.692
ARB/ACE-I	307 (95.6)	79 (95.2)	73 (93.6)	77 (96.2)	78 (97.5)	0.668
Statins	316 (98.4)	83 (100)	77 (98.7)	79 (98.8)	77 (96.2)	0.271

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between continuous and quartile-based Abeta40 levels and incident outcome events. Multivariable survival models for main endpoints were built under a bootstrap resampling procedure. Hazard ratios (HRs) and CI were expressed per 1-pg/ml Abeta40 increase as well as highest versus lowest Abeta40 quartile increment. We calibrated the multivariable survival models with the likelihood ratio test and the Akaike and Bayes information criteria. The incremental reclassification value of Abeta40 over conventional predictors of CV death was assessed by category-free continuous net reclassification improvement (NRI), categorized NRI (risk for CV death: low <6%, intermediate 6% to 9%, high >9%), and integrated discrimination improvement (IDI), which integrates the NRI over all possible cutoffs and is equivalent to the difference in discrimination slopes, over different models, using established risk factors. Lack of substantial population heterogeneity for all models assessing CV death was confirmed by adjustment for cohort origin in all multivariate models and by random-effects model used in pooled analysis. All tests were 2-tailed, and results were considered statistically significant for p values <0.05. All statistical analyses were performed using SPSS 21 (IBM Corporation, Armonk, New York) and Stata 11.1 (StataCorp LP, College Station, Texas). A detailed description of the statistical methodology is in the Online Appendix.

RESULTS

The study design is depicted in **Figure 1**. Associations between baseline characteristics of the 2 CHD cohorts in stable condition and quartiles of Abeta40 are shown in **Table 1**. Age, hsCRP level, hsTnT level, estimated glomerular filtration rate (eGFR), and left ventricular ejection fraction (LVEF) progressively increased among Abeta40 quartiles. The incidence of CV death was significantly increased at the highest Abeta40 quartile compared with the other quartiles in both CHD cohorts in stable condition ($p < 0.001$) (**Table 1**, **Figures 2A and 2B**). Similarly, the incidence of combined CV death and nonfatal acute myocardial infarction (MI) and incidence of the extended composite endpoint (CV death, nonfatal acute MI, rehospitalization due to ACS or myocardial revascularization) were also increased at the highest quartile of Abeta40 in both cohorts ($p < 0.001$) (**Table 1**). Abeta40 remained a significant independent predictor for CV death and MACE after multivariable adjustment for age, sex,

TABLE 1 Continued

	Overall	1st Quartile	2nd Quartile	3rd Quartile	4th Quartile	p Value*
Athens cohort (n = 556)						
Participants, n	556	139	139	139	139	
Median serum Abeta40, pg/ml (IQR)	36.4 (25.3)	15.5 (6)	28.9 (5.9)	41.2 (5.8)	80.1 (92.1)	<0.001
Mean age, yrs	62.2 (9.3)	60.6 (9.1)	62.7 (10)	62.4 (9.1)	62.9 (8.6)	0.153
Female	91 (16.5)	26 (18.7)	21 (15.1)	21 (5.3)	23 (16.7)	0.843
CV risk factors						
Hypertension	369 (66.4)	84 (60.4)	88 (63.3)	99 (71.2)	98 (70.5)	0.127
Diabetes mellitus	198 (35.6)	42 (30.2)	47 (33.8)	53 (38.1)	56 (40.3)	0.316
Smoking	158 (28.4)	33 (23.7)	45 (32.3)	42 (30.2)	38 (27.3)	0.394
Family history of CHD	198 (35.6)	52 (37.4)	47 (33.8)	47 (33.8)	52 (37.4)	0.724
Clinical parameters						
SBP, mm Hg	130 (19.5)	130 (22.5)	131.2 (16.6)	130 (20.5	130 (17.8)	0.942
Cholesterol, mg/dl	168 (40.3)	173 (36.8)	170 (43.4)	160 (41.6)	162 (38.7)	0.115
HDL cholesterol, mg/dl	42.6 (10.8)	45.2 (10.2)	43.7 (10.1)	41.5 (10.9)	40 (11.2)	<0.001
LDL cholesterol, mg/dl	96.4 (33.8)	99 (33.3)	98.7 (38.2)	96.4 (34.2)	91.6 (29.9)	0.244
BMI, kg/m^2	28.66 (4.64)	28.54 (3.96)	28.95 (5.81)	28.76 (4.13)	28.4 (4.43)	0.788
Median CRP, mg/l (IQR)	2.36 (4.92)	1.86 (3.30)	2.19 (3.63)	2.54 (5.23)	3.37 (7.60)	0.09
LVEF, %	50.6 (11.3)	52.7 (10.2)	51.6 (10)	50.6 (12)	47.7 (12.3)	0.006
Number of vessels						
1	208 (37.4)	61 (43.9)	58 (41.7)	52 (37.4)	37 (26.6)	0.12
2	185 (33.3)	38 (27.3)	46 (33.1)	48 (34.5)	53 (38.1)	
3	159 (28.6)	40 (28.8)	35 (25.2)	39 (28.1)	45 (32.4)	
CV endpoints						
CV death	55 (10.7)	7 (5.2)	13 (9.8))	13 (9.8)	22 (19.5)	0.004
CV death and AMI	116 (22.7)	21 (15.7)	22 (16.7)	39 (29.3)	34 (30.1)	0.004
Extended composite endpoint	198 (38.7)	46 (34.3)	38 (28.8)	61 (45.9)	53 (46.9)	0.006
CV drugs						
Antiplatelets (aspirin/clopidogrel)	487 (90.7)	129 (94.9)	120 (90.2)	121 (91.0)	117 (86.7)	0.143
Beta-blockers	422 (81.9)	107 (80.5)	102 (80.3)	105 (82.7)	108 (84.4)	0.805
ARB/ACE-I	383 (73.2)	92 (69.2)	94 (73.4)	98 (76.0)	99 (74.4)	0.634
Statins	407 (86.6)	106 (86.9)	104 (88.1)	94 (82.5)	103 (88.8)	0.491

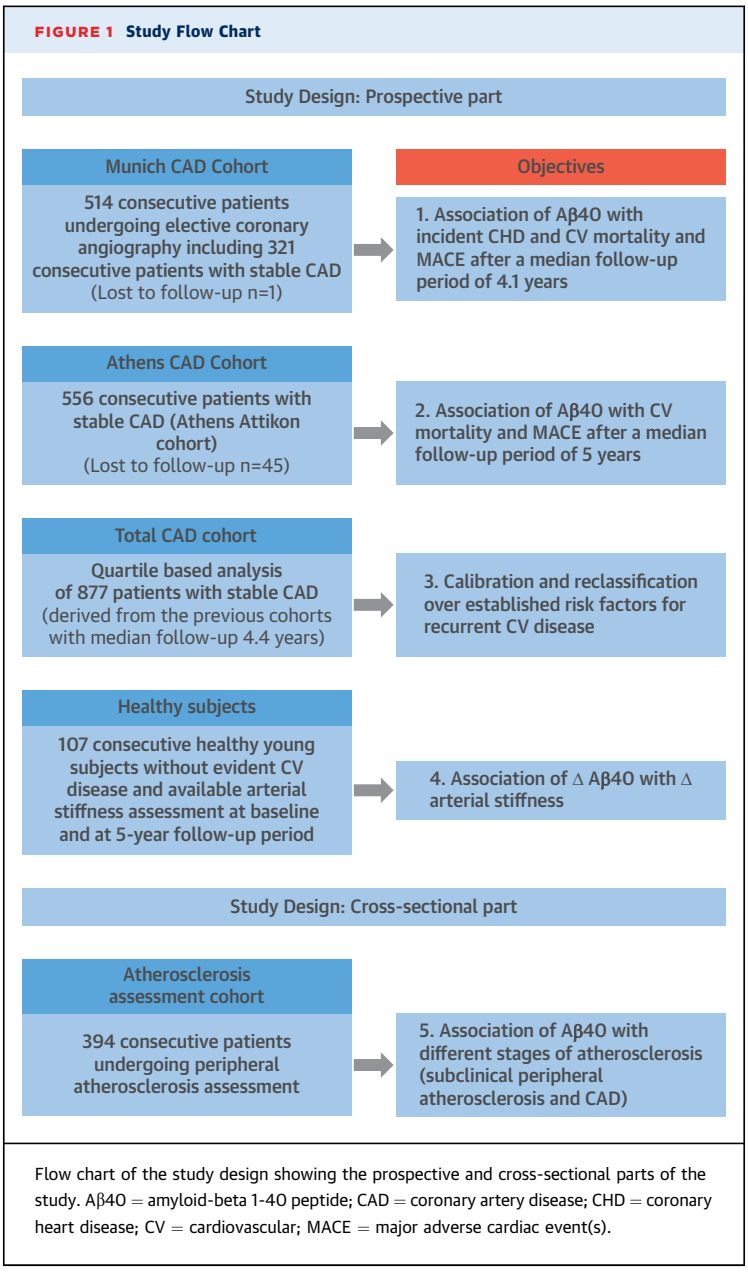
Values are n (%) or mean ± SD unless otherwise indicated. Abeta40 levels for the Munich cohort are expressed as mean ± SD and for the Athens cohort as median (IQR). *As derived from 1-way analysis of variance for continuous variables or chi-square test for nominal variables. The Kruskal-Wallis test was used for continuous variables deviating from normality.

Abeta40 = amyloid-β 1-40 peptide; ACE-I = angiotensin-converting enzyme inhibitor; AMI = acute myocardial infarction; ARB = angiotensin receptor blocker; BMI = body mass index; CAD = coronary artery disease; CHD = coronary heart disease; CRP = C-reactive protein; CV = cardiovascular; eGFR = estimated glomerular filtration rate; HDL = high-density lipoprotein; IQR = interquartile range; LDL = low-density lipoprotein; LVEF = left ventricular ejection fraction; SBP = systolic blood pressure.

LVEF, and hsCRP level (HR per 1-pg/ml increase for CV death in Munich cohort: 1.023; 95% CI: 1.007 to 1.039; $p < 0.05$; Athens cohort: 1.038; 95% CI: 1.018 to 1.057; $p < 0.001$) (**Table 2**). After additional adjustment for eGFR and hsTnT level, which were available for the Munich cohort, Abeta40 remained a significant independent predictor of all endpoints.

Taking into consideration that the statistical analysis in the individual cohorts offered similar results, we sought to assess whether Abeta40 levels improved model calibration for risk prediction and correctly reclassified risk for CV death and other CV events over the best predictive model. Because the number of adverse events per cohort was inadequate to perform such an analysis in each cohort, we created a combined CHD cohort from the quartiles of the Munich and Athens cohorts (n = 877). As described in the Online Appendix, we found no evidence of substantial heterogeneity between the 2 cohorts. Online Table 1 contains the descriptive characteristics of the combined total CHD cohort.

During the follow-up period of 4.4 years, patients experienced 77 CV deaths, 144 cumulative CV deaths and nonfatal acute MIs, and 257 MACE events in the total CHD cohort. In a univariate model, the cumulative incidence of CV death, as well as the secondary and extended composite endpoints, was significantly associated with increasing quartiles of Abeta40 (**Figure 2C**, Online Table 1). Abeta40 remained a significant predictor for CV death, as well as for secondary endpoints after multivariable adjustments for predictors of CV death (age, sex, LVEF, hsCRP level; HR of the highest quartile vs. lowest quartile: 3.02; 95% CI: 1.51 to 6.04; $p < 0.001$) (Online Table 2). After



Flow chart of the study design showing the prospective and cross-sectional parts of the study. Aβ40 = amyloid-beta 1-40 peptide; CAD = coronary artery disease; CHD = coronary heart disease; CV = cardiovascular; MACE = major adverse cardiac event(s).

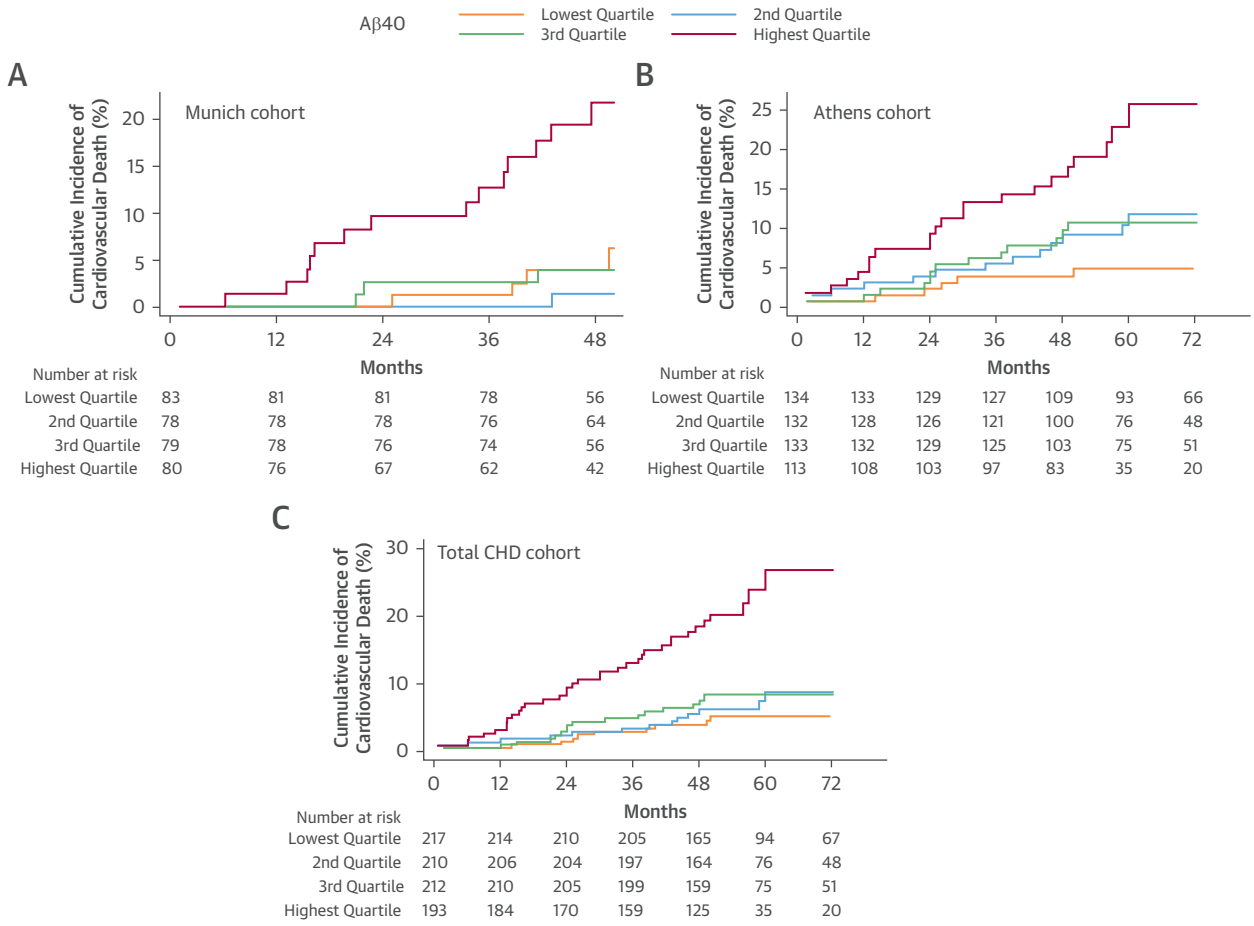
additional adjustment for traditional risk factors over the best predictive model, these results did not substantially change.

Similar associations were observed in an analysis using Abeta40 as a standardized continuous variable (Online Table 3). As shown in **Table 3**, Abeta40 significantly improved model calibration over the best predictive model for all 3 prospective endpoints (likelihood ratio test $p < 0.001$). Next, we addressed whether Abeta40 correctly reclassified risk of patients with stable CHD, thus predicting CV endpoints. Applying a category-free continuous NRI, category-based NRI for CV death (4.4-year risk categories <6%, 6% to 9%, and >9%), and IDI, we observed that Abeta40 correctly and significantly reclassified patients from lower to higher risk and vice versa better than age, sex, and LVEF (**Table 3**). Abeta40 improved reclassification (continuous NRI) even when hsCRP level was added to the adjustment model (NRI 36.7% for CV death; $p < 0.001$). Similarly, Abeta40 offered a significant IDI after adjustment for CV death predictors ($p < 0.001$). Similar results were obtained for CV death and nonfatal acute MI (secondary endpoint) and for the extended composite endpoint (**Table 3**). When the reclassification analysis was adjusted for cohort origin, these results did not change (data not shown). When reclassification analysis was performed for continuous standardized Abeta40, these results did not substantially change either (Online Table 4).

ARTERIAL STIFFNESS. Arterial stiffness measured by pulse-wave velocity (PWV) is a marker of early vascular disease progression. It is not known if Abeta40 is involved in arterial stiffening. Thus, we evaluated the possible association of Abeta40 with arterial stiffness progression (on the basis of PWV) over time. The descriptive characteristics of the 107 apparently healthy individuals, who were followed for 5 years, are shown in Online Table 5, and changes of PWV over time are graphically shown in Online Figure 1. At each visit (baseline and follow-up), detailed risk assessments and PWV measurements were performed. Abeta40 levels were significantly increased after 5-year follow-up (26.1 ± 18.5 pg/ml vs. 43.7 ± 25.3 pg/ml; $p < 0.001$). By linear mixed-model analysis, changes in plasma Abeta40 levels during this follow-up were independently associated with concomitant changes in calculated CV risk (Heart-score; $p = 0.008$) and PWV ($p = 0.006$) (Online Table 6) in the same direction.

To evaluate the association of Abeta40 with different stages of atherosclerosis in various arterial beds, we measured Abeta40 plasma levels in a cohort of 394 consecutive subjects who underwent peripheral atherosclerosis assessment (baseline characteristics in Online Table 7). Plasma Abeta40 levels significantly correlated with markers of subclinical atherosclerosis (**Figure 3**). After adjustment for traditional risk factors (age, sex or eGFR, arterial hypertension, hyperlipidemia, body mass index, smoking, diabetes mellitus), Abeta40 levels independently correlated with common carotid intima-media thickness (IMT) (odds ratio: 2.078; 95% CI: 1.089 to 3.966; $p = 0.027$), lower ankle brachial index (beta = −0.211; $p = 0.005$), and a higher probability for increased atheromatous burden in the carotid or

FIGURE 2 **Cumulative Probability of Death According to Quartile of Abeta40 Plasma Levels in Patients With CAD**



The Kaplan-Meier survival analysis shows that patients belonging to the highest quartile of Abeta40 had the highest probability of death compared with patients in other quartiles in the Munich **(A)** ($p < 0.001$) and Athens **(B)** ($p = 0.002$) cohorts, respectively. **(C)** The combined total cohort consists of all patients with CAD ($n = 877$) derived from the Munich and Athens cohorts. The Kaplan-Meier survival analysis shows that patients belonging to the highest quartile of Abeta40 had the highest probability of death compared with patients belonging to the other quartiles ($p < 0.001$). Abbreviations as in Figure 1.

femoral arteries as expressed by the number of plaques per patient ($p = 0.043$). Moreover, an increased burden of subclinical atherosclerosis, including increased IMT, involvement of at least 2 arterial beds or at least 3 peripheral sites with plaques, or presence of CHD, was associated with higher levels of Abeta40 compared with no evidence of subclinical atherosclerosis or minor involvement (Figure 3). After adjustment for traditional risk factors and eGFR as described above, these differences remained significant ($p < 0.05$).

Cross-sectional analysis of the Munich cohort also revealed that the incidence of angiographically confirmed CHD was positively associated with Abeta40 quartiles (Online Table 7). Abeta40 plasma concentration was higher in patients with CHD even after adjustment for traditional risk factors (age, sex, type 2 diabetes mellitus, smoking, hypertension, and hyperlipidemia; $p = 0.025$) or Framingham risk score ($p = 0.009$). After additional adjustment for hsCRP level, eGFR <60 ml/min/1.73 m^2, and hsTnT level, Abeta40 remained significantly associated with CHD ($p = 0.028$).

DISCUSSION

We investigated the clinical relevance of Abeta40 blood levels in 2 cohorts of patients with stable CHD who were followed for CV death and MACE for a median of 4.4 years. After adjustment for clinical predictors, Abeta40 was independently associated with the risk of CV death or MACE. The results were

TABLE 2 Univariate and Multivariate Analysis of Abeta40 as a Predictor of Study Endpoints

	HR per 1-pg/ml Increase (95% CI)	HR Lowest to Highest Quartile (95% CI)	HR Plot Lowest to Highest Quartile (95% CI)
CV death			
Munich cohort (n = 321)			
Univariate	1.027 (1.017–1.038)*	6.3 (2.7–15.1)*	
Model 1	1.024 (1.009–1.04)†‡	4.01 (1.59–10.1)†	
	1.02 (1.007–1.034)†§	3.57 (1.3–9.86)†§	
Model 2	1.024 (1.008–1.04)†‡	3.83 (1.53–9.63)†	
	1.021 (1.003–1.04)†‡§	3.54 (1.27–9.84)†§	
Model 3	1.023 (1.007–1.039)†‡	3.48 (1.36–8.89)†	
	1.021 (1.003–1.04)†‡§	3.34 (1.17–9.51)†§	
Athens cohort (n = 556)			
Univariate	1.037 (1.02–1.056)*	4.25 (1.81–9.97)*	
Model 1	1.038 (1.019–1.057)*	3.84 (1.64–9.02)†	
Model 2	1.041 (1.021–1.061)*	3.57 (1.51–8.4)†	
Model 3	1.038 (1.018–1.057)*	3.22 (1.36–7.65)†	
Secondary composite endpoint (CV death and/or AMI)			
Munich cohort			
Univariate	1.023 (1.013–1.032)*	5.12 (2.37–11.03)*	
Model 1	1.019 (1.006–1.032)†‡	3.64 (1.6–8.25)†	
	1.014 (1.002–1.026)†§	2.95 (1.2–7.25)†§	
Model 2	1.019 (1.006–1.033)†‡	3.49 (1.54–7.9)†	
	1.015 (1.00–1.03)‡§	2.92 (1.18–7.21)†§	
Model 3	1.019 (1.005–1.034)†‡	3.33 (1.44–7.68)†	
	1.015 (1.00–1.031)‡§	2.86 (1.13–7.19)†§	
Athens cohort			
Univariate	1.039 (1.024–1.055)*	2.73 (1.56–4.78)*	
Model 1	1.039 (1.023–1.054)*	2.71 (1.55–4.75)*	
Model 2	1.041 (1.025–1.057)*	2.66 (1.52–4.66)†	
Model 3	1.037 (1.022–1.053)*	2.55 (1.44–4.54)†	

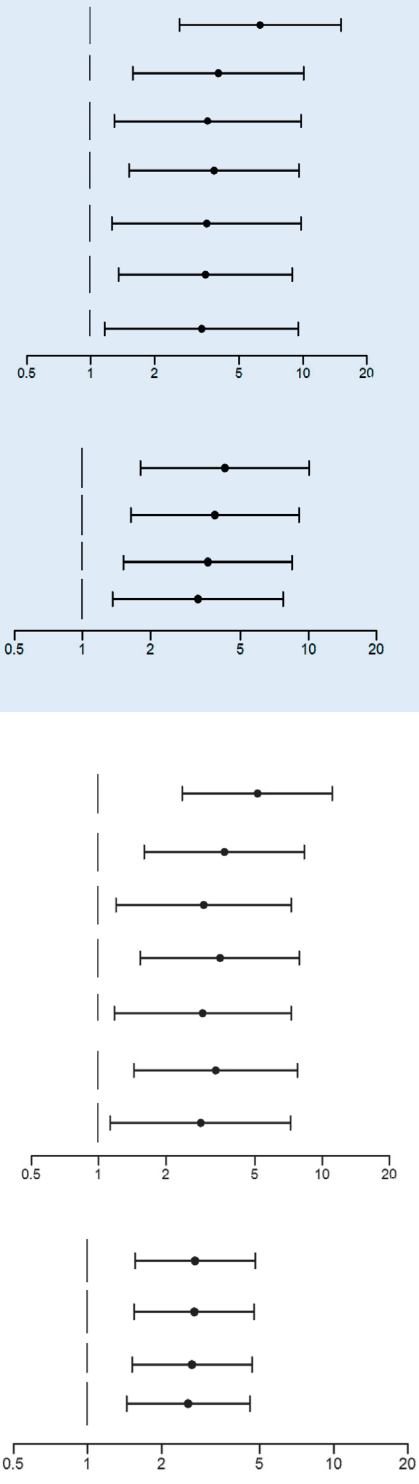


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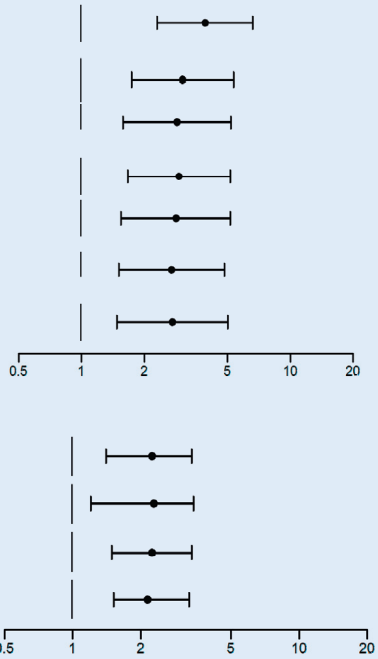
TABLE 2 Continued

	HR per 1-pg/ml Increase (95% CI)	HR Lowest to Highest Quartile (95% CI)	HR Plot Lowest to Highest Quartile (95% CI)
Extended composite endpoint (CV death, AMI, rehospitalization for ACS, coronary revascularization)			
Munich cohort			
Univariate	1.022 (1.015–1.03)*	3.91 (2.3–6.57)*	
Model 1	1.019 (1.011–1.027)*	3.05 (1.74–5.34)*	
	1.017 (1.008–1.027)*§	2.85 (1.58–5.14)†§	
Model 2	1.018 (1.01–1.027)*	2.92 (1.67–5.11)†	
	1.018 (1.008–1.027)*§	2.82 (1.55–5.11)†§	
Model 3	1.016 (1.005–1.027)†‡	2.69 (1.51–4.81)†	
	1.016 (1.006–1.026)†§	2.71 (1.48–4.97)†§	
Athens cohort			
Univariate	1.039 (1.026–1.053)*	2.24 (1.49–3.36)*	
Model 1	1.039 (1.026–1.052)*	2.28 (1.52–3.42)*	
Model 2	1.041 (1.028–1.055)*	2.24 (1.49–3.36)*	
Model 3	1.038 (1.025–1.052)*	2.14 (1.41–3.25)*	



Model 1: age and sex (or eGFR indicated by §) Model 2: age, sex (or eGFR indicated by §). and LVEF. Model 3: age, sex (or eGFR indicated by §), LVEF, and high-sensitivity CRP and high-sensitivity Troponin-T (available only in Munich cohort). eGFR was not inserted in models with age and sex because these parameters are included in its equation. *Observed statistical significance $p < 0.001$. †Observed statistical significance $p < 0.05$. ‡CI are calculated under resampling (bootstrapping with 1,000 replications).

ACS = acute coronary syndrome; HR = hazard ratio per 1-unit increase in Abeta40 or transition from lowest to highest quartile in Abeta40; other abbreviations as in Table 1.

virtually identical between the 2 cohorts. The current findings suggest that high levels of Abeta40 may help identify patients at increased risk of CV events. Further, we addressed the association of Abeta40 levels with subclinical atherosclerosis in 2 additional cohorts. We found that alterations in Abeta40 levels after 5-year follow-up were independently associated with aortic stiffness progression. Moreover, blood levels of Abeta40 were independently associated with the extent of arteriosclerotic disease, including arterial stiffness, incident IMT, incident peripheral arterial atherosclerotic plaques, and CHD.

Abeta40 plasma levels were associated with aging in all of the study cohorts, in accordance with previous reports (15). However, Abeta40 correlated with CV events independently of aging and eGFR, variables well known to affect its levels, reflecting the fact that a multifactorial regulatory network is responsible for Abeta40 clearance from blood including kidney, liver, and peripheral tissue degradation by a plethora of proteases (16). Abeta40 levels correlated with the presence of angiographic CHD as well as the presence, extent, and progression of subclinical peripheral atherosclerosis, confirming experimental evidence that APP and Abeta40 are critically involved in vascular inflammation. Abeta40 activates numerous vascular cells, leading to vascular inflammation, as depicted in the **Central Illustration**. Taking into consideration that Abeta40 was an independent predictor of changes in arterial stiffness after 5-year follow-up in a healthy cohort, this peptide may contribute to vascular aging and progression of preclinical arterial damage long before clinically evident CV disease is established. This finding concurs with recent evidence showing that arterial stiffness was associated with cerebral amyloid-beta deposition over a 2-year follow-up in elderly adults without dementia and implies that increased circulating Abeta40 levels may be the linking mechanism in this association (17). Given that arterial stiffness as an early marker of arteriosclerosis is closely associated with adverse CV events (18), it is tempting to hypothesize that Abeta40 also may predict MACE in the general population.

TABLE 3 Improvement in Model Calibration and Reclassification of Abeta40 Over Best Predictive Model for Endpoints

Calibration Parameters			Reclassification Parameters					
LR Test			Continuous NRI					IDI
	AIC (BIC)	LR Chi-Square (p Value)	Among Event Subjects	Among Nonevent Subjects	Overall (95% CI)*	p Value	NRI (SE)†	Coefficient (SE)
Primary endpoint (CV death; n = 77)								
Model 1	986.1 (995.5)	15.1 (<0.001)	35.06%	12.04%	47.1% (24–70.4)	<0.001	12% (5.9)‡	1.85 (0.57)§
+ Abeta40	973 (987.1)							
Model 2	969.5 (983.6)	14.2 (<0.001)	35.06%	9.1%	44.2% (24–70)	0.001	22.2% (7.1)‡	2.01 (0.64)§
+ Abeta40	957.3 (976.2)							
Model 3	956.7 (975.5)	11.9 (0.001)	27.28%	10.68%	36.7% (12.5–60.7)	0.001	22.4% (7)‡	1.92 (0.62)§
+ Abeta40	946.7 (970.3)							
Secondary composite endpoint (CV death and AMI; n = 144)								
Model 1	1,740 (1,750)	23.95 (<0.001)	29.16%	11.3%	40.5% (21.1–59.8)	<0.001	6.1% (3.1)‡	1.65 (0.45)§
+ Abeta40	1,718 (1,733)							
Model 2	1,735 (1,749)	23.86 (<0.001)	30.56%	9.84%	40.4% (21.9–58.9)	<0.001	13.2% (5.4)‡	1.67 (0.44)§
+ Abeta40	1,713 (1,732)							
Model 3	1,689 (1,708)	22.23 (<0.001)	30.5%	10.1%	40.4% (20.3–60.5)	<0.001	14.1% (5.4)‡	1.67 (0.45)§
+ Abeta40	1,669 (1,692)							
Extended composite endpoint (CC death, AMI, rehospitalization for ACS, coronary revascularization; n = 257)								
Model 1	3,000 (3,010)	28.6 (<0.001)	19.84%	9.86%	29.7% (15.3–44.1)	<0.001	7.2% (1.43)	1.62 (0.45)§
+ Abeta40	2,974 (2,988)							
Model 2	2,988 (3,002)	28.66 (<0.001)	19.04%	9.16%	29% (12.7–45.3)	<0.001	16.8% (5.7)‡	1.52 (0.43)§
+ Abeta40	2,961 (2,980)							
Model 3	2,913 (2,932)	25.49 (<0.001)	20.16%	8.3%	28.5% (12.4–44.5)	0.002	21% (5.6)§	1.36 (0.41)‡
+ Abeta40	2,890 (2,913)							

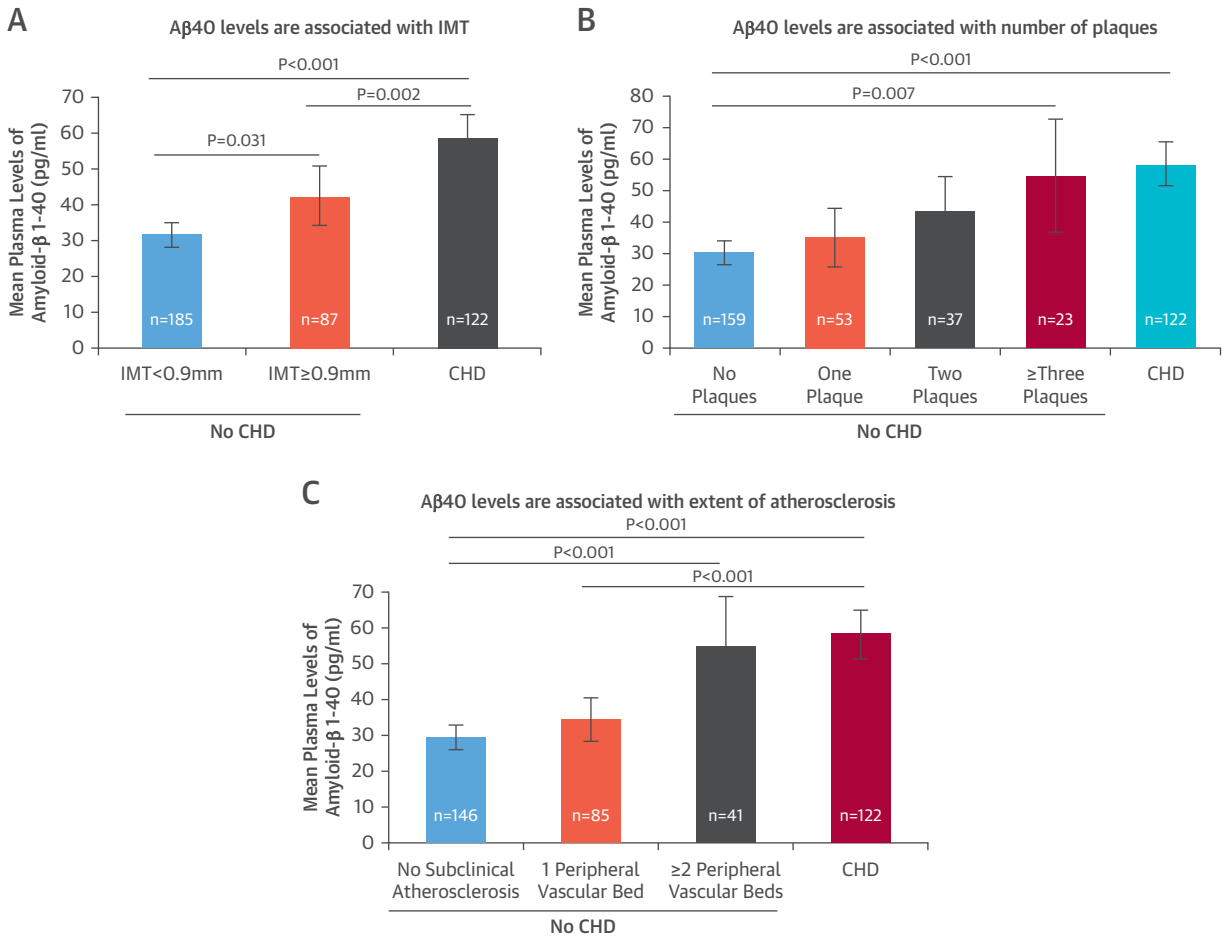
Model 1: age and sex. Model 2: age, sex, and LVEF. Model 3: age, sex, LVEF, and CRP. All models were built using the bootstrap resampling procedure. *95% CI are provided under resampling (bootstrapping with 1,000 replications). †Category-based NRI: CV death: low risk <6%, moderate risk 6% to 9%, high risk >9%; CV death and ACS: low risk <16%, moderate risk 16% to 20%, high risk >20%; extended composite endpoint: low risk <28%, moderate risk 28% to 35%, high risk >35%. ‡p < 0.05. §p < 0.001.

AIC = Akaike information criterion; BIC = Bayesian information criterion (AIC and BIC are unitless. Smaller values indicate better fit to data when compared with nested models.); IDI = integrated discrimination index; LR chi-square: twice the difference in log likelihoods between nested models under chi-square distribution with 1 degree of freedom; LR test = likelihood ratio test; NRI = net reclassification index; other abbreviations as in Tables 1 and 2.

CLINICAL IMPLICATIONS. Clinical guidelines underline the importance of novel biomarker development in specifically addressing the incremental diagnostic and prognostic benefit derived beyond that of established risk factors. Multiple pathways contribute to atherogenesis, atheroprogression, and plaque instability, leading ultimately to thrombosis. Current efforts focusing on biomarker development try to address risk stratification refinement by adding information from biomarkers that reflect distinct pathways involved in vascular inflammation complementing traditional predictive models. Our data indicated that patients in the highest Abeta40 quartile had a risk of CV death more than 3 times as great and a risk of MACE more than 2 times as great as patients in the lowest quartile. Interestingly, a nongraded response of Abeta40 in association with CV events was observed, being strongest in the highest quartile. Given that we found equally strong correlations with Abeta40 as a continuous variable and that we adjusted for all known commonly used confounders, this nongraded response may be attributed either to residual confounding of unknown factors or to specific biological attributes of Abeta40.

Our results suggest that a threshold concentration of Abeta40 (highest quartile) exists that differentiates patients with events, probably reflecting Abeta40 oligomer formation. Much more toxic to cells than Abeta40 monomers, Abeta40 oligomers are formed under conditions of Abeta40 monomer accumulation (19,20). Irrespective of response type, several mechanisms may explain a causal relationship of high Abeta40 levels with the ultimate step in cardiac events such as Abeta40-mediated platelet aggregation (21) and arrhythmogenesis (22), as well as release of matrix metalloproteinase 9 and increased plaque vulnerability (8,23). Using contemporary statistical techniques that are considered prerequisites for biomarker validation, such as model calibration and reclassification markers, we report here for the first time the use of Abeta40 as a tool for refining CV risk prediction in patients with stable CHD. Notably, this study provides the first evidence that higher Abeta40 levels were associated with a worse CV outcome in patients with stable CHD, even after adjustment for

FIGURE 3 **Abeta40 Plasma Levels and Subclinical Atherosclerosis and CHD**



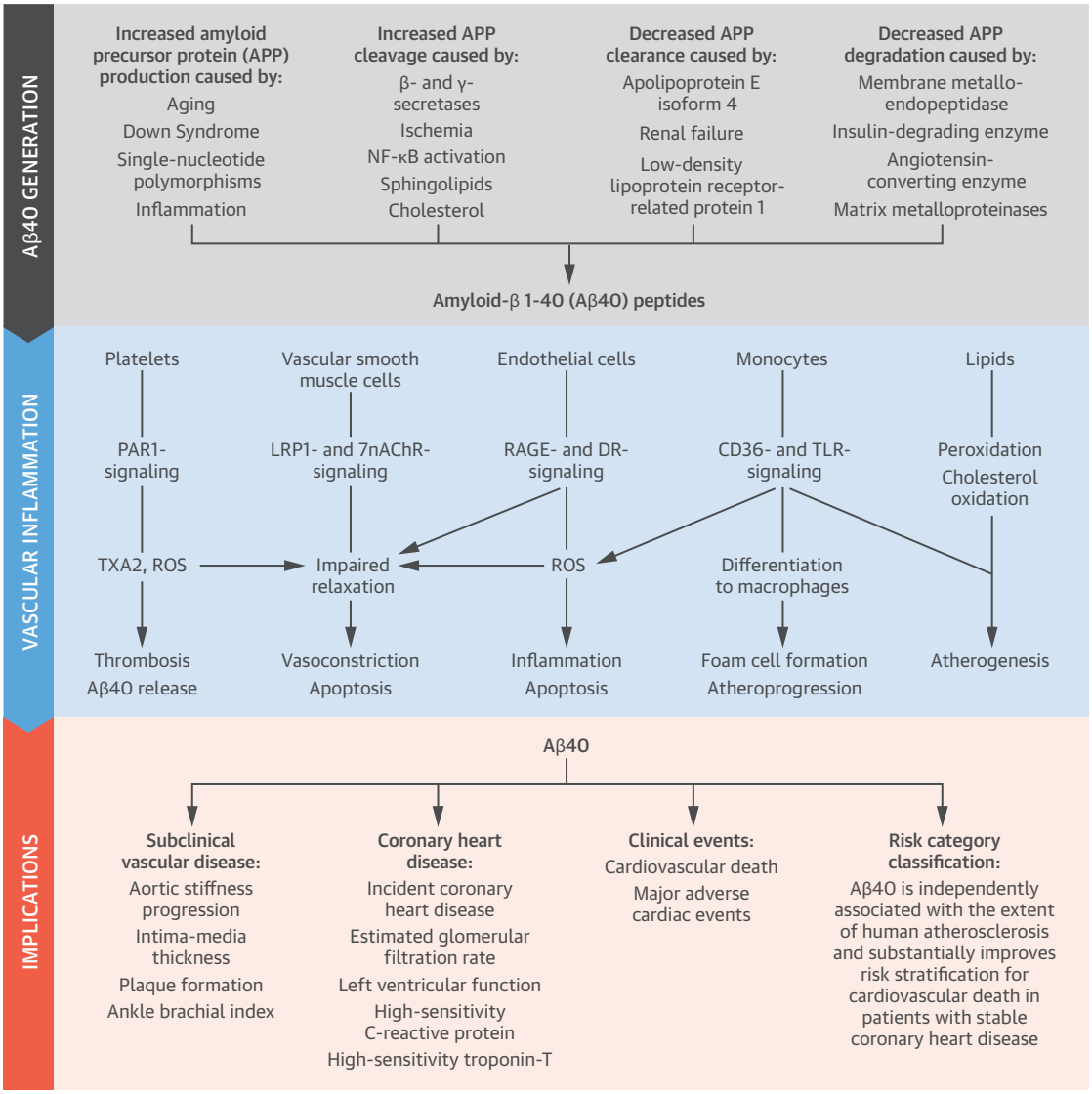
Compared with patients with stable CHD, mean plasma levels of Abeta40 rose **(A)** in subjects with increased common carotid intima-media thickness (IMT >0.9 mm), **(B)** across increasing number of atherosclerotic plaques in the carotid and femoral arteries, and **(C)** across increasing scores of atherosclerosis. All p values were calculated by analysis of variance with Bonferroni post-hoc tests among groups using log transformed Abeta40 values. Abbreviations as in Figure 1.

hsCRP, a well-established inflammatory biomarker. The assessment of Abeta40 as a biomarker may be useful for identifying subgroups who would benefit from additional diagnostic testing, an aggressive treatment of modifiable risk factors, or less intensive medical surveillance and therapy.

Our findings should trigger further research assessing Abeta40 as a potential therapeutic target for secondary CV prevention. Statins and antihypertensive therapy already have been shown to affect Abeta40 levels (24-27). Statins have reduced the production of Abeta40 in several cell culture and animal studies (25). Considering the detrimental effects of Abeta40 on CV disease, further studies are needed to assess whether statins exert their beneficial pleiotropic effects partially through regulation of Abeta40 production or clearance. Similarly, research is needed to assess the effect of some antihypertensive drugs, given that angiotensin receptor blockers may decrease Abeta40 concentrations in the brain (24,26), whereas furosemide, nitrendipine, and candesartan prevent oligomerization of Abeta40 in vitro (27).

STUDY LIMITATIONS. Circulating levels of Abeta40 were not comparable among the study cohorts, probably due to methodological differences in storage conditions, which are known to affect Abeta40 stability and final concentration (28,29). This limitation does not allow derivation of reference cutoff values for Abeta40 and poses difficulties in the joint assessment of the 2 CHD cohorts. We addressed this issue by using 2 distinct methods of pooling of the individual values (by quartiles and standardized continuous values).

CENTRAL ILLUSTRATION **Inflammatory Signaling Pathways of Abeta40**



Stamatelopoulos, K. et al. J Am Coll Cardiol. 2015; 65(9):904–16.

Amyloid-beta 1-40 peptide (Abeta40) production depends on: 1) production of amyloid precursor protein (APP); 2) cleavage of APP to Abeta peptides; 3) clearance; and 4) degradation mechanisms. Abeta40 binds to a plethora of receptors on the surface of cells and activates downstream pathways. For instance, binding of Abeta40 to platelet protease-activated receptor 1 (PAR1) induces downstream signaling affecting the thromboxane A2 (TXA2) signaling pathway, reactive oxygen species (ROS) production, platelet degranulation, and Abeta40 release, leading to platelet activation, aggregation, and thrombosis. Similarly, Abeta40 binding to smooth muscle cells or endothelial cells through appropriate receptors can ultimately lead to apoptosis along different pathways. Taken altogether, there is abundant experimental evidence supporting the notion that Abeta40 may play a critical role in all stages of atherosclerosis. 7nAChR = alpha-7 nicotinic receptor; DR = death receptor; LRP1 = low-density lipoprotein receptor-related protein 1; NF-κB = nuclear factor-kappaB; RAGE = receptor for advanced glycation end products; TLR = toll-like receptor.

Larger studies are needed to determine a cutoff value of Abeta40 levels in blood and its possible usage in clinical practice. Our results need to be further validated and should not be extrapolated to the general population without suspected or known CHD. Additional studies in community-based cohorts are likely to be valuable in determining the possible incremental prognostic value of Abeta40 for CV events.

Due to the limited sample size and number of CV deaths in each individual CHD cohort, we could not adjust for all biologically relevant risk factors in each individual CHD cohort (such as all traditional risk factors); rather, we used bootstrapping and adjusted for the best predictive model. Other potential limitations include residual confounding by unknown or coexisting conditions not ascertained at the time of blood sampling. We addressed this limitation by further adjusting the observed associations for all traditional risk factors over the best predictive model in the large combined CHD cohort. The consistently confirmed associations of Abeta40 with surrogates of CV risk and/or CV outcome and reclassification indexes in 3 independent prospective cohorts, as well as in the pooled cohort, strongly supports the clinical utility of Abeta40 as a biomarker for secondary prevention.

CONCLUSIONS

The present study demonstrated that Abeta40 was independently associated with the extent of human atherosclerosis and substantially improved risk stratification for CV death in patients with stable CHD. Because the proinflammatory effects of Abeta40 can be altered by established CV therapy, these findings may provide a rationale for a novel anti-inflammatory therapeutic target in patients with CHD. Whether treatment aimed at reducing the extent of Abeta40-induced vascular inflammation can positively alter the disease course remains to be determined.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: In patients without clinically overt coronary heart disease, blood levels of the proinflammatory peptide Abeta40 are associated with subclinical atherosclerosis and progression of arterial stiffness, independent of other conventional risk factors. Further, in patients with stable coronary heart disease, blood levels of Abeta40 are associated with cardiovascular mortality and morbidity over 5 years, independent of other conventional risk factors.

TRANSLATIONAL OUTLOOK: Further studies are warranted to validate the predictive value of measuring blood levels of Abeta40 in the general population, assess the effect of risk reduction therapies on blood levels of Abeta40, and test the safety and efficacy of specific anti-amyloid-beta therapies in patients with atherosclerosis.

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KEY WORDS arterial stiffness, biomarker, risk stratification, subclinical atherosclerosis

APPENDIX For supplemental tables and figures, please see the online version of this article.