JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes

ORIGINAL ARTICLE

Ultrasound analysis of gray-scale median value of carotid plaques is a useful reference index for cerebro-cardiovascular events in patients with type 2 diabetes

Kyoko Ariyoshi[1], Shigeru Okuya[2]*, Ichiro Kunitsugu[3], Kimie Matsunaga[1], Yuko Nagao[1], Ryuta Nomiyama[1], Komei Takeda[1], Yukio Tanizawa[1]

[1]Division of Endocrinology, Metabolism, Hematological Sciences, and Therapeutics, Department of Bio-Signal Analysis, Yamaguchi University Graduate of Medicine, Ube, [2]Health Administration Center, Yamaguchi University Organization for University Education, Yamaguchi, and [3]Department of Public Health, Yamaguchi University Graduate of Medicine, Ube, Japan

Keywords
Atherosclerosis, Eicosapentaenoic acid, Plaque echogenicity

***Correspondence**
Shigeru Okuya
Tel.: +81-83-933-5163
Fax: +81-83-933-5160
E-mail address: okuya@yamaguchi-u.ac.jp

J Diabetes Invest 2015; 6: 91–97

doi: 10.1111/jdi.12242

ABSTRACT

Aims/Introduction: Measurements of plaque echogenicity, the gray-scale median (GSM), were shown to correlate inversely with risk factors for cerebro-cardiovascular disease (CVD). The eicosapentaenoic acid (EPA)/arachidonic acid (AA) ratio is a potential predictor of CVD risk. In the present study, we assessed the usefulness of carotid plaque GSM values and EPA/AA ratios in atherosclerotic diabetics.

Materials and Methods: A total of 84 type 2 diabetics with carotid artery plaques were enrolled. On admission, platelet aggregation and lipid profiles, including EPA and AA, were examined. Using ultrasound, mean intima media thickness and plaque score were measured in carotid arteries. Plaque echogenicity was evaluated using computer-assisted quantification of GSM. The patients were then further observed for approximately 3 years.

Results: Gray-scale median was found to be a good marker of CVD events. On multivariate logistic regression analysis, GSM <32 and plaque score ≥5 were significantly associated with past history and onset of CVD during the follow-up period, the odds ratios being 7.730 (P = 0.014) and 4.601 (P = 0.046), respectively. EPA/AA showed a significant correlation with GSM (P = 0.012) and high-density lipoprotein cholesterol (P = 0.039), and an inverse correlation with platelet aggregation (P = 0.046) and triglyceride (P = 0.020). Although most patients with CVD had both low GSM and low EPA/AA values, an association of EPA/AA with CVD events could not be statistically confirmed.

Conclusions: The present results suggest the GSM value to be useful as a reference index for CVD events in high-risk atherosclerotic diabetics. Associations of the EPA/AA ratio with known CVD risk factors warrant a larger and more extensive study to show the usefulness of this parameter.

INTRODUCTION

Diabetes mellitus is a well-known risk factor for cerebro-cardiovascular disease (CVD)[1]. Evaluations of cerebro-cardiovascular complications are very important for predicting patient outcomes[1]. Because CVD develops as a result of vascular atherosclerotic changes, several methods have been developed to evaluate these atherosclerotic changes in the body. They include ultrasound analyses of the carotid arteries. Intima media thickness (IMT) and plaque scores (PS) have been widely used for assessing atherosclerotic changes representing CVD risk. More recently, the characteristics of plaque contents, such as echogenicity, have received research attention[2,3].

Received 17 April 2013; revised 30 January 2014; accepted 2 April 2014

Quantitative assessment of the echogenicity of plaques can be carried out by utilizing computer-assisted quantification of the gray-scale median (GSM)[3,4]. Plaques rich in calcium and fibrous tissue are more echogenic (higher GSM), whereas plaques containing abundant lipids and hemorrhagic components are more echolucent (lower GSM). Echolucent carotid plaques are more vulnerable and are associated with a higher risk of CVD. Patients with diabetes mellitus tend to have plaques with lower GSM; that is, the form of GSM correlated inversely with serum triglyceride (TG) levels[3]. In a community-based cohort study, carotid intima media-GSM was found to be an independent predictor of CVD mortality[5].

In contrast, increasing evidence suggests that consumption of n-3 polyunsaturated fatty acids (PUFAs) protects against CVD[6–8]. This beneficial effect of n-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) might occur independently of their lipid-lowering effects[9]. One potential mechanism leading to decreased CVD events is plaque stabilization through the anti-inflammatory effects of EPA. Meanwhile, some eicosanoids produced from arachidonic acid (AA) in the n-6 PUFA family have been reported to promote inflammatory and pro-thrombotic activities[10]. In addition, the EPA to AA ratio, an index drawn from these fatty acids, is reported to be lower in patients with CVD[8]. The Japan EPA Lipid Intervention Study (JELIS)[8], a large randomized controlled trial, has shown that purified EPA administration added to statin treatment provides a 19% reduction in major coronary events. JELIS has also shown that a low EPA/AA ratio can independently predict future CVD events in patients with a previous history of coronary artery diseases[11].

However, very few data are available about the relationship between serum PUFAs and carotid plaque stability, especially in patients with diabetes. Also, little is known about the association between GSM and the development of CVD in diabetic patients. Therefore, we assessed the usefulness of the carotid plaque GSM value and the EPA/AA ratio in atherosclerotic in patients with type 2 diabetes by examining the associations among carotid plaque characteristics, lipid profiles and CVD.

MATERIALS AND METHODS

Patients

This was a retrospective observational study designed to evaluate the correlations of GSM and the EPA/AA ratio with CVD events (at baseline and at the end of additional follow-up period). The patients received no other interventions during the entire study period. We both assessed cross-sectional data at baseline and carried out a longitudinal analysis (based on approximately 3 years of follow up) in the present study. In total, 84 consecutive type 2 diabetic patients with carotid artery plaques, who had been admitted to Yamaguchi University Hospital between June 2006 and July 2009, were enrolled. Informed consent was obtained from all patients. Those with cancer, renal dysfunction, liver disorders or receiving EPA treatment were excluded from the present study. All patients underwent carotid ultrasonography, laboratory tests, spontaneous platelet aggregation (PA) testing and lipid profile measurements including low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), TG, EPA and AA measurements. The plasma levels of EPA and AA in fasting blood samples were simultaneously analyzed in all cases using preserved serum from individual patients, by capillary gas chromatography at an external laboratory (SRL, Tokyo, Japan). Glycated hemoglobin (HbA1c), expressed in the National Glycohemoglobin Standardization Program units equivalent value[12], was measured by high-performance liquid chromatography. Participants were observed for an average of an additional 3 years and the data regarding subsequent CVD events were thereby collected. Myocardial infarction, angina and stroke (ischemic or hemorrhagic) were defined as CVD events. An acute myocardial infarction was diagnosed if a patient had typical chest pain with ST-segment elevation on electrocardiography and an increase in the plasma marker for myocardial infarction. Angina was diagnosed when patients had clear electrocardiographic changes or had already been diagnosed by a cardiologist. Stroke was diagnosed by typical symptoms and neuroimaging findings. The study protocol complied with the rules of the Helsinki Declaration, and was approved by our institutional ethics committee for human research.

Spontaneous Platelet Aggregation

As an indicator of pro-thrombotic states, spontaneous platelet aggregation (PA) was measured by evaluating the maximum percent decrease in optical density, and by assessing laser light scattering intensity using an AG10 system (KOWA, Tokyo, Japan). This method was previously described in detail by Ozaki *et al.*[13]

Carotid plaque imaging

A high-resolution carotid artery ultrasonographic examination was carried out in our ultrasound laboratory with a 10-MHz linear array transducer (SSD-α10; ALOKA, Tokyo, Japan). One experienced operator (the first author) carried out all of the carotid scans. The bilateral common carotid arteries (CCA), carotid bulbus and internal carotid arteries (ICA) in the neck were scanned. We defined mean IMT as the average IMT of three locations in the bilateral CCA. The three IMT determinations were carried out at the site with the thickest intima and two adjacent points (located 1 cm upstream and 1 cm downstream from the thickest site). Plaque was defined as a focal lesion with IMT ≥1.1 mm protruding into the lumen. PS were calculated by totaling the thickness of all plaques in the bilateral CCA, bulbus and ICA in each patient. Plaque echogenicity was assessed objectively by computer-assisted quantification of GSM values[4] using Adobe Photoshop software (CS3; Adobe System, San Jose, CA, USA). The normalized calibration curve for the gray-scale was prepared from 0 for blood to 195 for adventitia. In patients with more than one plaque, the most echolucent plaque was chosen by visual assessment.

Statistical Analysis

Associations of individual GSM values or the EPA/AA ratio with the clinical characteristics of patients were assessed using Pearson's correlation coefficient. Associations of clinical events with carotid arterial echo characteristics and other atherosclerotic risk factors were analyzed using logistic regression. In consideration of the sample number and data distribution, our data were subjected to logistic regression analysis using boundary or cut-off values. Based on the international and the Asian consensus, we included the following boundary values in the models as well as continuous variables; age (≥60 years), body mass index (BMI; ≥25), hypertension (≥140 and/or 90 mmHg), HbA1c (≥7.0%) and dyslipidemia (LDL-C ≥140 mg/dL or HDL-C <40 mg/dL or TG ≥200 mg/dL). The established cut-off values; that is, those for EPA/AA (≥0.5), GSM (<32) and IMT (≥1.0 mm), were obtained from previous reports[14–16]. Furthermore, receiver operating characteristic (ROC) analysis was carried out to determine the optimal cut-off points for PA and PS to acquire the R^2 score of the logistic regression analysis, with routine adjustment for other risk confounders[17,18]. Thus, we estimated the optimal cut-off points associated with CVD events in our patients as follows; PA (≥20,000 V) and PS (≥5.0 mm). Furthermore, to determine whether or not whole increments of each crude parameter would be associated with event onset, similarly to binominal-categorized variables, we also confirmed the associations between continuous variables for risk factors and CVD events using monovariate and multivariate logistic regression models. Additionally, we calculated mono- and multivariate regression models including each risk factor as a continuous variable to investigate the associations between these risk factors and GSM or EPA/AA. In multivariable logistic regression analyses, we included the following 13 variables: age (≥60 years), sex, BMI (≥25), smoking, hypertension (≥140 and/or 90 mmHg), HbA1c (≥7.0%), dyslipidemia (LDL-C ≥140 mg/dL or HDL-C <40 mg/dL or TG ≥200 mg/dL), no medication, EPA/AA ratio (≥0.5), GSM (<32), PA (≥20,000), mean IMT (≥1.0 mm) and PS (≥5.0 mm). We statistically limited certain variables for confounder adjustment in the models, as further discussed in the Results section. In order to confirm the validity of these variables, we also applied the stepwise selection method. Results from logistic models are expressed as odds ratios (ORs) with 95% confidence intervals (CIs). The Kaplan–Meier method was used to estimate event-free rates after division into groups for analysis of GSM, EPA/AA or PS. The log–rank test was used to assess differences in survival curves derived from the Kaplan–Meier analysis. Statistical significance was defined as a value of $P < 0.05$. The statistical analyses were carried out using SPSS version 19.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Patients' baseline characteristics are listed in Table 1. A total of 48 of the 84 enrolled patients were male, and the average age was 61.4 ± 10.7 years. This group of patients was slightly overweight (mean BMI 25.1 ± 5.2 kg/m^2). As to past history related to CVD, the reported numbers of myocardial infarction, angina, ischemic stroke and hemorrhage stroke events were 8, 2, 3 and 0, respectively. All of these events had occurred within 9 years of the admission date serving as the starting point of the present study. Because these patients were hospitalized for the purpose of diabetic control, the average HbA1c value was 9.3 ± 2.2% despite various medications being administered. The mean spontaneous PA value was 22,239 ± 19,940 V. In contrast, blood pressure and lipid levels that were essentially within the target values had been obtained by prior treatments.

The mean IMT and average PS were 0.796 ± 0.243 and 6.055 ± 4.462 mm, respectively. The average GSM in this group was 50.2 ± 35.5.

During the approximately 3-year follow-up period, there were two myocardial infarction events (including one recurrence) and four ischemic strokes (including one recurrence).

In the present study, 15 patients with 'no medication' did not have previous CVD events and no CVD events occurred during the study period. For this parameter, the standard error was too large (approximately 9,259) and the OR had the

Table 1 | Baseline patient characteristics

No. of patients	84
Male, *n* (%)	48 (57.1)
Age (years)	61.4 ± 10.7
Body mass index (kg/m^2)	25.1 ± 5.2
Clinical history	
Myocardial infarction, *n* (%)	8 (9.1)
Angina, *n* (%)	2 (2.3)
Ischemic stroke, *n* (%)	3 (3.5)
Risk factor	
Smoking, *n* (%)	42 (50.0)
Hypertension; on medication, *n* (%)	40 (47.6)
Hypercholesterolemia; on medication, *n* (%)	34 (40.4)
No medication, *n* (%)	15 (17.8)
Laboratory findings	
HbA1c (%)	9.3 ± 2.2
LDL-C (mg/dL)	119.8 ± 37.8
HDL-C (mg/dL)	52.1 ± 14.3
Triglycerides (mg/dL)	154.2 ± 88.3
Platelet aggregation (V)	22,239 ± 19,940
EPA/AA ratio	0.36 ± 0.18
Blood pressure	
Systolic (mmHg)	123.5 ± 17.4
Diastolic (mmHg)	73.0 ± 9.7
Echo data	
Common carotid mean IMT (mm)	0.796 ± 0.243
Carotid plaque score (mm)	6.055 ± 4.462
GSM	50.2 ± 35.5

AA, arachidonic acid; EPA, eicosapentaenoic acid; GSM, gray-scale median; HDL-C, high-density lipoprotein-cholesterol; IMT, intima media thickness; LDL-C, low-density lipoprotein-cholesterol. Data are shown as *n* (%) or means ± standard deviation.

extraordinary value of '0' (data not shown). Meanwhile, smoking, confirmed to be a significant risk factor of CVD events, was strongly related to both PS and sex ($R = 0.342$, $P < 0.01$ and $R = 0.433$, $P < 0.01$, respectively).

To evaluate risk factors without multicolinearity, we analyzed 11 (of the 13) variables, as follows: age (≥60), sex (female), BMI (≥25), hypertension (≥140/90), HbA1c (≥7.0), dyslipidemia (LDL-C ≥140 or HDL-C <40 or TG ≥200), EPA/AA ratio (≥0.5), GSM (<32), PA (≥20,000), mean IMT (≥1.0) and PS (≥5.0). Multivariate logistic regression analysis identified GSM <32 and PS ≥5 as variables showing a statistically significant association with CVD events both at baseline and during the additional 3-year follow-up period, the OR being 7.730 ($P = 0.014$) and 4.601 ($P = 0.046$), respectively (Table 2). In contrast, BMI ≥25, HbA1c ≥7.0, dyslipidemia (LDL-C ≥140, HDL-C <40 or TG ≥200), hypertension, EPA/AA ratio <0.5, PA ≥20,000 and IMT ≥1.0 showed no correlation with CVD events. We confirmed that the CV-models yielded results similar to those of binomially-categorized variables (data not shown).

In further analysis with stepwise selection, 79.8% of all CVD episodes in the enrolled patients were explained by the existence of only GSM<32 and PS ≥5 (likelihood ratio = 68.923). After adjustment for smoking, GSM <32 and PS ≥5.0 both appeared to be significantly associated with CVD events.

We then evaluated the associations of the EPA/AA ratio and GSM, individually, with various atherosclerosis risk factors (Tables 3 and 4). The EPA/AA ratio in single regression analysis correlated positively with age and HDL-C, and negatively with both TG and spontaneous PA. The EPA/AA ratio in multiple regression analysis correlated positively with age only (Table 3). There was no association between the EPA/AA ratio and either mean IMT or PS. In contrast, we recognized a significant association between EPA/AA and GSM in single regression analysis ($r = 0.271$, $P = 0.012$; Tables 3 and 4, Figure 1). However, the GSM in single and multiple regression analyses showed no associations with LDL-C, HDL-C, TG, PA, mean IMT, PS or the EPA/AA ratio (Table 4). CVD events were more frequent in the lower GSM and lower EPA/AA groups. In Figure 1, the number of CVD cases in the 'low GSM (<32) + low EPA/AA (<0.5)', 'low GSM + high EPA/AA (≥0.5)', 'high GSM (≥32) + low EPA/AA' and 'high GSM + high EPA/AA' areas were eight, four, four and one, respectively (shown as black triangles, squares and circles in Figure 1). To verify the association between EPA/AA (<0.5 or ≥0.5) and GSM (<32 or ≥32) stratified according to CVD events, we applied the Mantel–Haenszel χ^2-test. Within a small group of just 17 CVD cases, there was no significant association between EPA/AA and GSM ($\chi^2 = 0.302$, $P = 0.528$). However, the EPA/AA ratio was significantly associated with GSM in 67 patients without CVD episodes and in the total of 84 patients ($\chi^2 = 8.408$, $P = 0.004$ and $\chi^2 = 4.942$, $P = 0.026$, respectively). The association trend was also significant between the EPA/AA ratio and GSM (Somers $D = -0.242$, trend P-value = 0.015).

Table 2 | Multivariate logistic regression analysis for cerebro-cardiovascular events

	No. of patients with CVD/total (17/84)	OR	95% CI	*P*-value
Age (years)				
<60	4/29	1 (Reference)	0.256–6.871	0.737
≥60	13/55	1.325		
Sex				
Male	8/48	1 (Reference)	0.672–9.547	0.170
Female	9/36	2.533		
BMI				
<25	7/36	1 (Reference)	0.113–2.123	0.340
≥25	10/48	0.490		
Hypertension				
No	16/68	1 (Reference)	0.098–3.185	0.511
Yes	1/16	0.558		
HbA1c				
<7.0	1/11	1 (Reference)	0.090–7.639	0.868
≥7.0	16/73	1.829		
Dyslipidemia				
No	7/42	1 (Reference)	0.219–3.698	0.845
Yes	10/42	0.901		
EPA/AA ratio				
<0.5	12/59	1 (Reference)	0.199–4.530	0.948
≥0.5	5/25	0.949		
GSM				
≥32	5/52	1 (Reference)	1.511–39.540	0.014
<32	12/32	7.730		
PA (V)				
<20,000	10/52	1 (Reference)	0.159–2.986	0.619
≥20,000	7/32	0.690		
Mean IMT (mm)				
<1.0	13/70	1 (Reference)	0.235–6.538	0.801
≥1.0	4/14	1.239		
PS (mm)				
<5.0	3/42	1 (Reference)	1.026–20.629	0.046
≥5.0	14/42	4.601		

AA, arachidonic acid; CI, confidence interval; CVD, cerebro-cardiovascular disease; EPA, eicosapentaenoic acid; GSM, gray-scale median; IMT, intima-media thickness; OR, odds ratio; PA, platelet aggregation; PS, plaque score. Dyslipidemia is defined as low-density lipoprotein cholesterol ≥140 mg/dL or high-density lipoprotein cholesterol <40 mg/dL or triglycerides ≥200 mg/dL.

When the patients were divided into two groups based on GSM values, patients with lower GSM (<32) experienced CVD events more frequently than those with higher GSM (≥32) during the 3-year follow-up period ($P = 0.025$; GSM <32: $n = 5$ vs GSM ≥32: $n = 1$), as shown by the Kaplan–Meier plots (Figure 2). Furthermore, when the patients were divided into two groups based on PS, patients with higher PS (≥5.0) experienced CVD events more frequently than those with lower PS (<5.0) during the follow-up period ($P = 0.011$; PS <5.0: $n = 0$

Table 3 | Single and multiple regression analyses of cerebro-cardiovascular disease-related risk factors for eicosapentaenoic acid/arachidonic acid ratio

Variable	*r*	*P*-value	β′	*P*-value
Age (years)	0.395	0.00019	0.305	0.013
LDL-C (mg/dL)	–0.063	0.567	0.077	0.480
HDL-C (mg/dL)	0.225	0.039	0.105	0.368
TG (mg/dL)	–0.252	0.020	–0.151	0.198
Platelet aggregation (V)	–0.246	0.046	–0.092	0.373
Mean IMT (mm)	0.126	0.251	0.088	0.475
PS (mm)	0.074	0.498	–0.004	0.977
GSM	0.271	0.012	0.207	0.053

GSM, gray-scale median; HDL-C, high-density lipoprotein-cholesterol; IMT, intima media thickness; LDL-C, low-density lipoprotein-cholesterol; PS, plaque score; TG, triglycerides.

Table 4 | Single and multiple regression analyses of cerebro-cardiovascular disease-related risk factors for gray-scale median

Variable	*r*	*P*-value	β′	*P*-value
Age (years)	0.130	0.237	0.164	0.219
LDL-C (mg/dL)	–0.030	0.780	0.028	0.806
HDL-C (mg/dL)	0.194	0.076	0.088	0.483
TG (mg/dL)	–0.069	0.527	0.090	0.473
Platelet aggregation (V)	–0.117	0.287	–0.074	0.503
Mean IMT (mm)	–0.176	0.109	–0.193	0.141
PS (mm)	–0.157	0.153	–0.117	0.392
EPA/AA ratio	0.271	0.012	0.235	0.053

Abbreviations are the same as in Tables 1 and 2. AA, arachidonic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein-cholesterol; IMT, intima media thickness; LDL-C, low-density lipoprotein-cholesterol; PS, plaque score TG, triglycerides.

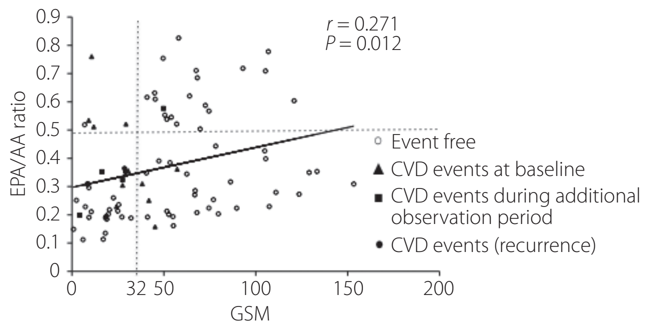

Figure 1 | Correlation between eicosapentaenoic acid (EPA)/arachidonic acid (AA) ratio and gray-scale median (GSM). White circles indicate patients without cerebro-cardiovascular disease (CVD). Black triangles represent those with CVD events at baseline. Black squares and black circles are patients who developed CVD events for the first time during the additional 3 years of observation, or as recurrences, respectively.

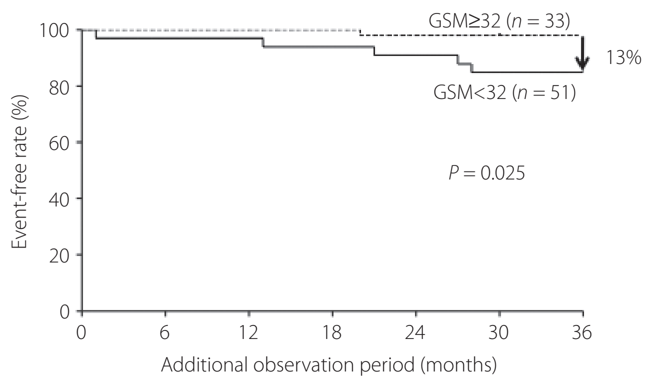

Figure 2 | Kaplan–Meier survival estimates of the incidence of cerebro-cardiovascular events in patients with gray-scale median (GSM) <32 (solid line) and GSM ≥32 (dashed line).

vs PS ≥5.0: *n* = 6), as shown by the Kaplan–Meier plots (Figure 3).

DISCUSSION

Our present results show that GSM analysis of carotid plaques is useful as a reference index for CVD events in diabetics, and that the plasma EPA/AA ratio might serve as a useful parameter for assessing high-risk diabetics.

It is believed that high lipid contents indicate plaque instability and vulnerability. Among carotid ultrasound evaluations, GSM, a new marker of lipid content and plaque vulnerability, is potentially useful for predicting CVD events. In fact, plaques with GSM <32 (echolucent) are reportedly associated with cerebral infarction[15]. The present study also showed a correlation between lower GSM and newly-occurring events by Kaplan–Meier analysis. Higher PS (PS ≥5), another marker of CVD risk on ultrasound evaluation, was also associated with CVD events in our patients. In contrast, no association was observed between mean IMT and CVD events. PS might be a better predictor of CVD events than mean IMT, because it is an index derived by evaluating more extensive regions including the CCA, bulbus and ICA, whereas mean IMT assessment is restricted to the CCA[19]. Overall, the present results suggest the nature of plaques (particularly plaque stability) to be represented by GSM, and that an index of the total numbers and thickness of plaques (PS) is a better reference index for CVD events than a simple marker of arterial wall-thickness (IMT).

The present study patients had fairly good lipid profiles with appropriate treatments. Half of the patients had been prescribed lipid-lowering agents. This explains why dyslipidemia was not associated with CVD events in the present study.

A previous study found that the average EPA/AA ratio in 60-year-old Japanese subjects was approximately 0.5[14]. Thus, we divided the enrolled patients into two groups, based on a cut-off of 0.5, to assess the effects of EPA/AA on CVD events.

Another important finding of the single regression analysis in the present study is that the lower EPA/AA ratio was significantly associated with higher TG, lower HDL-C, lower GSM

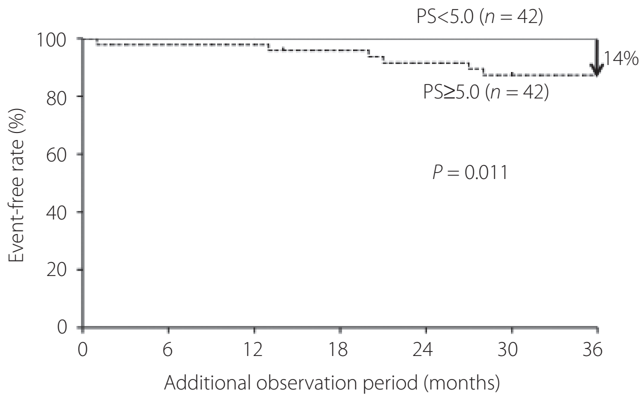

Figure 3 | Kaplan–Meier survival estimates of the incidence of cerebro-cardiovascular events in patients with plaque score (PS) ≥5.0 (dashed line) and PS <5.0 (solid line).

and a high spontaneous PA value, all established risk factors for CVD events in patients with type 2 diabetes. An imbalance in the ratio of PUFAs might also be implicated in the development of cardiovascular diseases, and the EPA/AA ratio could thus be a useful marker for detecting diabetic cardiovascular complications. Unfortunately, however, the EPA/AA ratio showed no significant associations with established risk factors for CVD in a multiple regression model and there were no significant associations with CVD in our multiple logistic analyses. The absence of significant effects could be as a result of the relatively small scale and short observation periods of the present study. Therefore, our findings warrant further evaluation of the EPA/AA ratio in larger, more extended studies. It is noteworthy that the plasma EPA/AA ratio can easily be measured using blood samples, such that the assay could be outsourced to commercial laboratories if necessary.

Based on the present study and previous reports, ultrasound determination of the GSM values of carotid plaques provides a useful reference index for cardiovascular events and can serve as an assessment parameter for high-risk diabetic patients. However, it has not yet been determined whether the relationship between the EPA/AA ratio and CVD development is significant.

The present data showed low GSM to be a potential predictor of cardiovascular events and that a low EPA/AA ratio might be a useful marker predicting future cardiovascular events in high-risk diabetics. This was an essentially observational study, and the number of patients was too small and the follow-up period too short to verify the usefulness of the EPA/AA ratio or to adequately examine its relationships with cardiovascular events. Given the limited samples, so-called classical risk factors, such as BMI, HbA1c and blood pressure, could not be regarded as CVD risk variables in the model for our multivariate analysis. Therefore, a large-scale prospective study needs to be carried out to resolve these issues.

ACKNOWLEDGMENT

The authors have no potential conflicts of interest to disclose.

REFERENCES

1. Haffner SM, Lehto S, Rönnemaa T, *et al.* Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998; 339: 229–234.
2. De Blois J, Stranden E, Jogestrand T, *et al.* Echogenicity of the carotid intima-media complex and cardiovascular risk factors. *Clin Physiol Funct Imaging* 2012; 32: 400–403.
3. Ostling G, Hedblad B, Berglund G, *et al.* Increased echolucency of carotid plaques in patients with type 2 diabetes. *Stroke* 2007; 38: 2074–2078.
4. Saberai MM, Tegos TJ, Nicolaides AN, *et al.* Reproducibility of computer-quantified carotid plaque echogenicity: can we overcome the subjectivity? *Stroke* 2000; 31: 2189–2196.
5. Wohlin M, Sundström J, Andrén B, *et al.* An echolucent carotid artery intima-media complex is a new and independent predictor of mortality in an elderly male cohort. *Atherosclerosis* 2009; 205: 486–491.
6. Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N Engl J Med* 1985; 312: 1205–1209.
7. Tavani A, Pelucchi C, Negri E, *et al.* n-3 Polyunsaturated fatty acids, fish, and nonfatal acute myocardial infarction. *Circulation* 2001; 104: 2269–2272.
8. Yokoyama M, Origasa H, Matsuzaki M, *et al.* Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomized open-label, blinded endpoint analysis. *Lancet* 2007; 369: 1090–1098.
9. Leaf A, Albert CM, Josephson M, *et al.* Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. *Circulation* 2005; 112: 2762–2768.
10. Allayee H, Roth N, Hodis HN. Polyunsaturated fatty acids and cardiovascular disease: implications for nutrigenetics. *J Nutrigenet Nutrigenomics* 2009; 2: 140–148.
11. Matsuzaki M, Yokoyama M, Saito Y, *et al.* Incremental effects of eicosapentaenoic acid on cardiovascular events in statin-treated patients with coronary artery disease. *Circ J* 2009; 73: 1283–1290.
12. The Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus, Seino Y, Nanjo K, Tajima N, *et al.* Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Invest* 2010; 1: 212–228.
13. Ozaki Y, Satoh K, Yatomi Y, *et al.* Detection of platelet aggregates with a particle counting method using light scattering. *Anal Biochem* 1994; 218: 284–294.
14. Yanagisawa N, Shimada K, Miyazaki T, *et al.* Polyunsaturated fatty acid levels of serum and red blood cells in apparently healthy Japanese subjects living in an urban area. *J Atheroscler Thromb* 2010; 17: 285–294.

15. El-Barghouty N, Geroulakos G, Nicolaides A, *et al*. Computer-assisted carotid plaque characterisation. *Eur J Vasc Endovasc Surg* 1995; 9: 389–393.
16. Chambless LE, Heiss G, Folsom AR, *et al*. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987–1993. *Am J Epidemiol* 1997; 146: 483–494.
17. Huang YC, Ho CC, Lin PT, *et al*. Optimal cutoff value of high-density lipoprotein cholesterol for predicting coronary artery disease in Taiwanese population. *Nutr Res* 2010; 30: 21–26.
18. Misra A, Vikram NK, Gupta R, *et al*. Waist circumference cutoff points and action levels for Asian Indians for identification of abdominal obesity. *Int J Obes* 2006; 30: 106–111.
19. O'Leary DH, Polak JF, Kronmal RA, *et al*. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. *N Engl J Med* 1999; 340: 14–22.