**Aims:** The ratio of eicosapentaenoic acid (EPA) to arachidonic acid (AA) is related to major adverse events and death in cardiovascular diseases. The association between long-term prognosis of ischemic stroke and EPA/AA ratio has not been clarified.

**Methods:** Acute ischemic stroke patients who had undergone blood examinations for polyunsaturated fatty acids were enrolled. Major cardiovascular events, including recurrence of ischemic stroke, occurrence of cardiovascular and peripheral artery diseases and hemorrhagic stroke, and death, were analyzed, retrospectively. Cox proportional hazards regression analysis was used to explore factors, including clinical characteristics, laboratory data including EPA/AA ratio, and treatments associated with major cardiovascular events and death.

**Results:** A total of 269 patients (mean age, 70 ± 13 years; 179 men) were enrolled. During follow-up (mean, 2.3 ± 1.0 years), 64 patients exhibited major cardiovascular events and death (annualized rate, 10.5% per person-year). Multivariate Cox analysis revealed that EPA/AA ratio (hazard ratio, 0.26; 95% confidence interval, 0.07–0.99; \( p = 0.048 \)) and statin therapy (hazard ratio, 0.43; 95% confidence interval, 0.25–0.73; \( p = 0.002 \)) correlated inversely with major cardiovascular events and death. In the Kaplan–Meier analysis, cumulative event-free rates were significantly lower among patients with EPA/AA ratio <0.33 and patients without statin therapy (\( p = 0.006 \)).

**Conclusions:** Low EPA/AA ratio at baseline and treatment without statins could predict mortality, recurrent ischemic stroke, cardiovascular and peripheral artery diseases, and hemorrhagic stroke among patients with acute ischemic stroke. The combination of baseline EPA/AA ratio and statin therapy could be critical in predicting the long-term prognosis of ischemic stroke patients.

**Key words:** Stroke, Eicosapentaenoic acid, Statins, Major cardiovascular events, Polyunsaturated fatty acids

---

**Introduction**

Stroke is the fourth most common cause of death in Japan and a major cause of disability worldwide. Another important issue is that the recurrence of ischemic stroke and comorbidity with cardiovascular diseases are not uncommon\(^1\). To date, several biomarkers such as C-reactive protein, interleukin 6, and brain natriuretic peptide have proven useful for predicting stroke recurrence, cardiovascular diseases, and death in ischemic stroke patients\(^2, 3\).

Polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA) are poorly synthesized in the human body. Intake of EPA is well-known to reduce the incidence of cardiovascular and cerebrovascular diseases\(^4, 5\). The protective roles against cardiovascular disease attributed to n-3 PUFAs could be due to interactions of a number of pleiotropic effects, including anti-inflammatory, anti-atherogenic, anti-
inflammatory, anti-platelet, anti-oxidant, vasodilatory and hypotensive properties, improvements in endothelial function, and favorable changes in lipid profiles. Importantly, plasma PUFA levels, particularly the EPA/AA ratio, have been associated with major adverse cardiovascular events among patients with cardiovascular and peripheral artery diseases. Emerging data have linked EPA/AA ratio to mortality among patients with heart failure. EPA/AA ratio has thus been suggested as a candidate marker for major adverse events and death in patients with cardiovascular diseases. To date, the association between long-term prognosis of ischemic stroke patients and plasma PUFA levels has remained essentially unknown.

**Aim**

The present study explored the association between EPA/AA ratio and long-term prognosis in ischemic stroke patients.

**Methods**

**Study Subjects**

Participants in the present study were obtained based on an analysis of data acquired from a prospective registry of 377 patients with acute ischemic stroke within 7 days of onset, with results available from analyses of plasma PUFA levels, and who had been admitted to the Department of Neurology at the Juntendo University Hospital between January 2014 and February 2016. Acute brain infarction was diagnosed as an acute neurological event lasting more than 24 h and associated with focal hyperintense lesions on diffusion-weighted imaging (DWI). Subtype of brain infarction was based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria. Transient ischemic attack was defined as a sudden onset of a focal neurological deficit suspected to be of cerebrovascular origin, lasting less than 24 h. This registry included comprehensive data on patient characteristics, atherosclerotic risk factors, radiological findings, and laboratory findings, including PUFA level at baseline, but excluded patients with stroke after cardiac surgery, patients who were already hospitalized and had received hospital meals for ≥7 days, patients who had received intravenous hyperalimentation, and patients who had been treated with n-3 PUFA formulations prior to stroke. Follow-up data were obtained by reviewing medical records, including major cardiovascular events defined as recurrence of ischemic stroke, hemorrhagic stroke, cardiovascular events (acute myocardial infarction, angina pectoris, and aortic diseases) and peripheral artery disease, and death since onset of acute ischemic stroke from our hospital, in addition to information on the use of antiplatelet or anticoagulant agents, statins, and other medications during the observation period. Those treatments were carried out in our hospital according to our best medical judgment and were not randomized. We enrolled patients for whom follow-up data were available for at least 6 months after discharge. Intervals between stroke onset and the day of major cardiovascular events and death were obtained for patients showing such events, and the date of last follow-up available for patients without major cardiovascular events and death was obtained from a review of medical records, referring to a previously described method. Patients who died within 60 days after stroke onset because of malignant tumor were excluded. This study was conducted in accordance with the Declaration of Helsinki. The independent ethics committee at the Juntendo University Hospital approved this study. We used clinical information obtained from medical records, and the need to obtain written informed consent from each patient was therefore waived in this retrospective study. The study protocol and the informed consent statement were provided on the website of our institution.

**Risk Factors**

Atherosclerotic vascular risk factors were defined as follows: 1) hypertension, history of using antihypertensive agents, systolic blood pressure >140 mmHg, or diastolic blood pressure >90 mmHg at 14 days after stroke onset; 2) diabetes mellitus, use of oral hypoglycemic agents or insulin, or glycosylated hemoglobin A1c ≥6.5%; 3) dyslipidemia, use of antihyperlipidemic agents, serum low-density lipoprotein cholesterol ≥140 mg/dL, high-density lipoprotein cholesterol <40 mg/dL, or triglycerides ≥150 mg/dL; 4) current smoking; 5) coronary artery disease, defined as a history of angina pectoris or myocardial infarction; or 6) peripheral artery disease, defined as a history of peripheral artery disease.

**MRI Sequences**

Magnetic resonance imaging (MRI) was performed using 1.5-T scanners during hospitalization. Diagnosis of acute brain infarction was based on a finding of focal hyperintensity not attributed to normal anisotropic diffusion or magnetic susceptibility artifacts on DWI. MRI sequences included axial DWI, fluid-attenuated inversion recovery imaging (FLAIR), and magnetic resonance angiography (MRA). DWI (repetition time (TR), 4156–4900 ms; echo time (TE), 60–89 ms) was used to assess the size and distribution of index stroke lesions. FLAIR (TR,
9000–10,000 ms; TE, 114–120 ms) was used to evaluate the degree of periventricular hyperintensity (PVH) in accordance with Fazekas grades 0–3. MRA (TR/TE, 19–26; TE, 2.8–6.9 ms) was used to detect intracranial stenosis >50%.

**Measurements of PUFA Levels**

Plasma levels of PUFAs including EPA, DHA, and AA were assayed by gas chromatography at an external laboratory (SRL Inc., Tokyo, Japan). Blood examinations were carried out within 24 h of admission or with the referral to the Department of Neurology in patients who developed ischemic stroke during hospitalization.

**Statistical Analysis**

Numerical values are reported as means ± standard deviation. Data were analyzed using the chi-square test for categorical variables and the Mann–Whitney U test for nonparametric analyses. Hazard ratios (HRs) for major cardiovascular events and death were obtained using multivariable Cox proportional hazard analysis. After univariate analysis of all clinically relevant covariates, those with a value of p < 0.2 were included in the multivariable Cox model, and a backward elimination (likelihood ratio) strategy was used. The Kaplan–Meier method and the log-rank test were employed to estimate cumulative event rates of major cardiovascular events and death, using EPA/AA ratio < 0.33 as the cut-off value according to a previous report. A two-sided p-value < 0.05 was considered significant. All data were analyzed using SPSS for Windows version 15.0 statistical software (SPSS, Chicago, IL).

**Results**

Among 377 patients with acute ischemic stroke who were admitted between January 2014 and February 2016, complete therapy data could not be obtained in 5 patients, and 74 patients were lost during follow-up; 29 patients were excluded because of death within 60 days after discharge, mainly owing to malignant neoplasms, and final data were successfully obtained for 269 patients (mean age, 70 ± 13 years; 179 men). Based on TOAST criteria, patients showed the following stroke subtypes: small artery occlusion in 36 patients, large artery atherosclerosis in 40 patients, cardioembolism in 67 patients, other determined etiology in 67 patients, and other undetermined etiologies in 28 patients. Transient ischemic attack was seen in 31 patients. During follow-up (mean, 2.3 ± 1.0 years), 27 patients experienced recurrent ischemic stroke, 7 showed cardiovascular disease, and 3 experienced hemorrhagic stroke. Twenty-seven patients died, including 2 deaths from cardiovascular disease, 10 from pneumonia, 8 from cancer, 2 from sepsis, 1 from liver cirrhosis, 1 from acute pancreatitis, and 3 from unknown causes. The incidence of the composite end point was 10.5% per person-year. The incidence of major cardiovascular events including recurrent ischemic stroke, hemorrhagic stroke, and cardiovascular and peripheral artery diseases was 6.1%, and the incidence of death was 4.4% per person-year.

**Clinical Characteristics of Patients with Major Cardiovascular Events and Death**

In 64 patients with major cardiovascular events and death, 2 had small artery occlusion, 15 had large artery atherosclerosis, 15 had cardioembolism, 22 had another determined etiology, 6 had other undetermined etiologies, and 4 patients experienced transient ischemic attack. We compared clinical characteristics and radiological and laboratory data according to the presence of major cardiovascular events and death (Table 1). Age was higher in patients with major cardiovascular events and death (p < 0.001). Hypertension, coronary artery diseases, peripheral artery diseases, and previous history of ischemic stroke were more common among patients with major cardiovascular events and death (p < 0.001). On laboratory data, levels of low-density lipoprotein cholesterol (LDL-C) were lower and levels of D-dimer were higher in patients with major cardiovascular events and death (p < 0.001 each). On laboratory data, levels of low-density lipoprotein cholesterol (LDL-C) were lower and levels of D-dimer were higher in patients with major cardiovascular events and death (p < 0.001 each). EPA levels were higher (74.9 ± 42.3 µg/mL vs 60.1 ± 33.4 µg/mL, p = 0.006), and ratios of EPA/AA and DHA/AA (0.41 ± 0.23 vs 0.35 ± 0.22, p = 0.015 and 0.81 ± 0.27 vs 0.74 ± 0.23, p = 0.046, respectively) were lower in patients with major cardiovascular events and death. During the clinical course, statin therapy was uncommon in patients with major cardiovascular events and death (66% vs 52%, p = 0.049).

**Factors Associated with Major Cardiovascular Events and death**

Table 2 shows that age, body mass index (BMI), hypertension, coronary artery diseases, previous history of stroke, NIHSS, PVH grade, LDL-C, EPA, DHA, EPA/AA ratio, and statin therapy correlated with major cardiovascular events and death in univariate Cox regression analyses (p < 0.05). In addition, current smoking, peripheral artery diseases, glucose levels, DHA/AA ratio, and anticoagulant therapy were
and death (HR, 0.86; 95% confidence interval [CI], 0.79–0.94;  \( p = 0.001 \)). Among PUFAs-associated factors, only EPA/AA ratio exhibited a significant inverse association with major cardiovascular events and death (Model C; HR, 0.26; 95%CI, 0.07–0.99;  \( p = 0.048 \) (Table 3), whereas EPA (Model A; HR, 0.99; 95%CI, 0.93–1.07;  \( p = 0.777 \))

Table 1. Baseline characteristics and radiological and laboratory findings at baseline according to classification by outcomes

| Characteristics                                      | Total       | Major cardiovascular events and death |       |
|------------------------------------------------------|-------------|---------------------------------------|-------|
|                                                      |  \( n = 69 \) | Absent \( n = 205 \)  | Present \( n = 64 \)  |       |
| Sociodemographic                                     |             |                                       |       |
| Age, years, mean ± SD                                | 70 ± 13     | 68 ± 14  | 76 ± 11  | <0.001 |
| Gender, male, no. (%)                                | 179 (67)    | 138 (67)  | 41 (64)  | 0.455  |
| Body mass index                                       | 23.3 ± 4.0  | 23.6 ± 4.1  | 22.2 ± 3.4  | 0.111  |
| Risk factors, no. (%)                                |             |                                       |       |
| Hypertension                                         | 192 (71)    | 136 (66)  | 56 (88)  | 0.001  |
| Diabetes mellitus                                    | 71 (26)     | 51 (25)  | 20 (31)  | 0.261  |
| Dyslipidemia                                         | 174 (65)    | 134 (65)  | 40 (63)  | 0.712  |
| Current cigarette smoking                            | 61 (23)     | 51 (25)  | 10 (16)  | 0.077  |
| Atrial fibrillation                                  | 51 (19)     | 37 (18)  | 14 (22)  | 0.419  |
| Coronary artery disease                              | 35 (13)     | 17 (8)  | 17 (27)  | <0.001 |
| Peripheral artery disease                            | 9 (3)       | 5 (2)  | 4 (6)  | 0.031  |
| Previous history of ischemic stroke                  | 41 (15)     | 24 (12)  | 17 (27)  | 0.008  |
| NIHSS score on admission, mean ± SD                  | 3.8 ± 5.2   | 2.9 ± 4.0  | 6.6 ± 7.2  | <0.001 |
| Transient ischemic attack, no. (%)                   | 31 (12)     | 27 (13)  | 4 (6)  | 0.197  |
| MRI                                                  |             |                                       |       |
| PVH, grade 0-3                                       | 1.0 ± 0.8   | 0.8 ± 0.8  | 1.3 ± 0.7  | <0.001 |
| Intracranial artery stenosis, no. (%)                | 62 (23)     | 46 (22)  | 16 (25)  | 0.801  |
| Laboratory findings, mean ± SD                       |             |                                       |       |
| LDL-C, mg/dL                                         | 111.9 ± 35.5 | 115.7 ± 32.5 | 99.5 ± 41.5 | <0.001 |
| HDL-C, mg/dL                                         | 51.9 ± 15.8 | 52.3 ± 15.6 | 50.5 ± 16.5 | 0.176  |
| Hemoglobin A1c, %                                    | 6.0 ± 1.1   | 6.0 ± 1.1  | 6.2 ± 1.1  | 0.101  |
| D-dimer, µg/mL                                       | 2.5 ± 4.5   | 1.8 ± 1.5  | 4.6 ± 8.5  | <0.001 |
| Glucose, mg/dL                                       | 121.9 ± 46.3 | 119.6 ± 46.2 | 129.2 ± 46.4 | 0.079  |
| AA, µg/mL                                            | 186.6 ± 49.5 | 188.1 ± 50.6 | 181.7 ± 46.0 | 0.467  |
| EPA, µg/mL                                           | 71.4 ± 40.8 | 74.9 ± 42.3 | 60.1 ± 33.4 | 0.006  |
| DHA, µg/mL                                           | 142.7 ± 44.9 | 146.6 ± 46.3 | 130.4 ± 37.5 | 0.467  |
| EPA/AA                                               | 0.40 ± 0.23 | 0.41 ± 0.23  | 0.35 ± 0.22  | 0.015  |
| DHA/AA                                               | 0.80 ± 0.27 | 0.81 ± 0.27  | 0.74 ± 0.23  | 0.046  |
| Treatment, n (%)                                     |             |                                       |       |
| Anti-platelet agents                                 | 188 (70)    | 147 (72)  | 41 (64)  | 0.249  |
| Anti-coagulant agents                                | 86 (32)     | 60 (29)  | 26 (41)  | 0.097  |
| Statins                                              | 168 (62)    | 135 (66)  | 33 (52)  | 0.049  |
| n-3 PUFAs formulations                               | 12 (4)      | 8 (4)  | 4 (6)  | 0.655  |

Chi-square test, the Mann-Whitney U test were used for comparison. NIHSS = National Institutes of Health stroke scale, MRI = magnetic resonance imaging, PVH = periventricular hyperintensity, LDL-C = low-density lipoprotein-cholesterol, HDL-C = high-density lipoprotein-cholesterol, AA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, PUFAs = Polyunsaturated fatty acids.

entered into the multivariate Cox regression analysis as covariates. EPA, DHA, EPA/AA ratio, and DHA/AA ratio were entered in Models A, B, C, and D, respectively, as these covariates confounded each other. Multivariate Cox regression revealed that BMI was negatively associated with major cardiovascular events and death (Model C; HR, 0.26; 95%CI, 0.07–0.99;  \( p = 0.048 \) (Table 3), whereas EPA (Model A; HR, 0.99; 95%CI,
Kaplan–Meier analysis was employed to explore both EPA/AA ratio and treatment without statins as predictors of major cardiovascular events and death, among 81 patients with EPA/AA ratio ≥ 0.33. Statin therapy was inversely correlated with major cardiovascular events and death in all models (HR, 0.43; 95%CI, 0.25–0.73; p=0.002) (Table 3).

Table 2. Univariate analysis of baseline characteristics, and radiological and laboratory findings predicting major cardiovascular events and death

| Characteristics                        | HR   | 95% CI     | p     |
|----------------------------------------|------|------------|-------|
| Sociodemographic                       |      |            |       |
| Age, per 1-year increase               | 1.06 | 1.03–1.09  | <0.001|
| Gender, male                           | 0.87 | 0.52–1.45  | 0.601 |
| Body mass index                        | 0.9  | 0.84–0.97  | 0.005 |
| Risk factors                           |      |            |       |
| Hypertension                           | 3.09 | 1.47–6.48  | 0.003 |
| Diabetes mellitus                      | 1.3  | 0.76–2.2   | 0.337 |
| Dyslipidemia                           | 0.84 | 0.50–1.39  | 0.487 |
| Current cigarette smoking              | 0.56 | 0.28–1.09  | 0.089 |
| Atrial fibrillation                    | 1.36 | 0.75–2.46  | 0.307 |
| Coronary artery disease                | 2.88 | 1.65–5.02  | <0.001|
| Peripheral artery disease              | 1.95 | 0.71–5.37  | 0.196 |
| Previous history of ischemic stroke    | 2.05 | 1.18–3.55  | 0.011 |
| NIHSS score on admission               | 1.09 | 1.06–1.12  | <0.001|
| MRI                                    |      |            |       |
| PVH, per 1-grade increase              | 1.76 | 1.35–2.3   | <0.001|
| Intracranial artery stenosis           | 1.17 | 0.66–2.05  | 0.594 |
| Laboratory findings                    |      |            |       |
| LDL-C                                  | 0.99 | 0.98–0.99  | 0.001 |
| HDL-C                                  | 0.99 | 0.98–1.01  | 0.474 |
| Hemoglobin A1c                         | 1.11 | 0.92–1.35  | 0.283 |
| Glucose                                | 1    | 1.00–1.01  | 0.133 |
| D-dimer                                | 1.07 | 1.04–1.09  | <0.001|
| AA                                     | 1    | 0.99–1     | 0.331 |
| EPA                                    | 0.99 | 0.98–1     | 0.014 |
| DHA                                    | 0.99 | 0.99–1     | 0.014 |
| EPA/AA ratio                           | 0.29 | 0.09–0.99  | 0.048 |
| DHA/AA ratio                           | 0.42 | 0.16–1.12  | 0.082 |
| Treatment                              |      |            |       |
| Anti-platelet agents                   | 0.74 | 0.44–1.23  | 0.247 |
| Anti-coagulant agents                  | 1.53 | 0.93–2.52  | 0.095 |
| Statins                                | 0.58 | 0.35–0.94  | 0.027 |
| n-3 PUFAs formulations                 | 1.68 | 0.61–4.63  | 0.314 |

NIHSS = National Institutes of Health Stroke Scale, MRI = magnetic resonance imaging, PVH = periventricular hyperintensity, LDL-C = low-density lipoprotein-cholesterol, HDL-C = high-density lipoprotein-cholesterol, AA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, PUFAs = polyunsaturated fatty acids.

0.99–1.00; p=0.140), DHA (Model B; HR, 1.00; 95%CI, 0.99–1.01; p=0.537), and DHA/AA ratio (Model D; HR, 0.31; 95%CI, 0.09–1.06; p=0.062) were unrelated to major cardiovascular events and death. Statin therapy was inversely correlated with major cardiovascular events and death in all models (HR, 0.43; 95%CI, 0.25–0.73; p=0.002) (Table 3).
with statin treatment, 48 patients with EPA/AA ratio \( \geq 0.33 \) without statin treatment, 87 patients with EPA/AA ratio \(< 0.33 \) with statin treatment, and 53 patients with EPA/AA ratio \(< 0.33 \) without statin treatment. Cumulative event-free rates were significantly lower in the order of patients with EPA/AA ratio \(< 0.33 \) and without statin therapy, with EPA/AA ratio \(< 0.33 \) and with statin therapy, with EPA/AA ratio \( \geq 0.33 \) and without statin therapy, and with EPA/AA ratio \( \geq 0.33 \) and with statin therapy (Fig. 1; log-rank test, \( p = 0.006 \)).

### Discussion

The present study explored the associations of PUFAs with long-term prognosis in patients with ischemic stroke. Our results showed that EPA/AA ratio among PUFA-associated factors was an independent predictor of major cardiovascular events such as recurrent ischemic stroke, hemorrhagic stroke, cardiovascular and peripheral artery diseases, and death. As important as this PUFA-associated factor, treatment without statins during follow-up correlated with major cardiovascular events and death.

According to large-scale epidemiological studies, intake of food enriched with EPA, DHA, and fish oil reduces the incidence of cardiovascular diseases and stroke\(^4\)\(^-\)\(^6\). Treatment with EPA formulations in addition to low-dose statins also significantly reduces coronary artery diseases as well as stroke compared with statin therapy alone\(^7\). More importantly, serum EPA/AA ratios at baseline were associated with major adverse events in coronary and peripheral artery diseases and increasing size of aortic arch aneurysm\(^1\)\(^,\)\(^1\)\(^2\)\(^,\)\(^1\)\(^9\).

#### Table 3. Factors associated with major cardiovascular events and death

| Characteristics                        | Multivariate Cox Regression |
|----------------------------------------|----------------------------|
|                                        | HR | 95% CI          | \( p \) |
| Age, per 1-year increase               | 1.04 | 1.01-1.07     | 0.021 |
| Body mass index                        | 0.86 | 0.79-0.94     | 0.001 |
| Hypertension                           | 2.24 | 0.98-5.15     | 0.056 |
| Current cigarette smoking              | 0.72 | 0.34-1.51     | 0.382 |
| Coronary artery disease                | 1.60 | 0.80-3.20     | 0.181 |
| Peripheral artery disease              | 0.64 | 0.20-2.04     | 0.449 |
| Previous history of ischemic stroke    | 1.41 | 0.74-2.71     | 0.297 |
| NIHSS score on admission               | 1.07 | 1.03-1.11     | 0.001 |
| PVH, per 1-grade increase              | 1.34 | 0.94-1.90     | 0.103 |
| LDL-C                                  | 0.99 | 0.98-1.00     | 0.179 |
| Glucose                                | 1.01 | 1.00-1.01     | 0.053 |
| D-dimer                                | 1.05 | 1.02-1.08     | 0.001 |
| EPA/AA ratio                           | 0.26 | 0.07-0.99     | 0.048 |
| Anti-coagulant agents                  | 1.43 | 0.81-2.54     | 0.219 |
| Statins                                | 0.43 | 0.25-0.72     | 0.002 |

NIHSS = National Institutes of Health Stroke Scale, PVH = periventricular hyperintensity, LDL-C = low-density lipoprotein-cholesterol, EPA = eicosapentaenoic acid, AA = arachidonic acid.

![Fig. 1. Kaplan–Meier curves of freedom from major cardiovascular events and death during follow-up](image-url)

The X axis indicates time in days since inclusion in the study. The Y axis indicates the proportion of patients surviving free of major cardiovascular events, including recurrence of ischemic stroke or occurrence of cardiovascular or peripheral artery disease or hemorrhagic stroke or death. Cumulative event-free rates were compared among patients: with EPA/AA ratio \(< 0.33 \) without statin therapy; with EPA/AA ratio \(< 0.33 \) with statin therapy; with EPA/AA ratio \( \geq 0.33 \) without statin therapy; and with EPA/AA ratio \( \geq 0.33 \) with statin therapy (\( p = 0.006 \) on log-rank test).
On the other hand, EPA/AA ratio was linked with cardiac mortality in heart failure and mortality from cancer and all-cause of mortality in normal subjects. These data suggest that the EPA/AA ratio may offer an important marker for not only major cardiovascular events but also death from any cause. Regarding cerebrovascular diseases, a relationship between EPA/AA ratio and early neurological deterioration has been shown. To the best of our knowledge, this study offers the first demonstration that EPA/AA ratio at baseline can predict long-term prognosis including major cardiovascular events and death among patients with acute ischemic stroke.

EPA, which has a structure comprising a 20-carbon chain with five double bonds, inhibits inflammatory eicosanoid mediators such as leukotrienes, prostaglandins, and thromboxanes induced by AA. Moreover, EPA has potent anti-inflammatory, anti-oxidant, and antiatherogenic effects on endothelial cells. Particularly in carotid atherosclerotic plaques, EPA also decreases the accumulation of inflammatory cytokines such as tumor necrosis factor-α, interleukin 6, and matrix metalloproteinase 1/2. Ischemic stroke patients with low EPA/AA ratio at baseline might thus have underlying pathogenesises such as chronic inflammation, oxidative stress, and potential systemic atherosclerosis in individuals, which contributed to recurrent stroke, cardiovascular and peripheral artery diseases, and death over the long term.

Statins are well-known to show benefits in terms of stroke prevention, and a meta-analysis has revealed that LDL-C reduction by 1 mmol/L (39 mg/dL) due to statin therapy decreases the risk of stroke by 21.1%. In particular, the Stroke Prevention by Aggressive Reduction in Cholesterol Levels trial showed beneficial results in the prevention of recurrent ischemic stroke. Current guidelines recommend intensive lipid-lowering effects via statins for patients with ischemic stroke with atherosclerotic origin and with LDL-C ≥ 100 mg/dL. Our data showed that the baseline level of LDL-C was 99.5 ± 41.5 mg/dL among patients with major cardiovascular events and death, and the use of statin therapy correlated inversely with major cardiovascular events and death, suggesting that the treatment without statins for ischemic stroke patients with even when LDL-C <100 mg/dL might be linked with major cardiovascular events and death. Importantly, the Kaplan–Meier analysis revealed that patients with low EPA/AA ratio <0.33, even with statin treatment, more frequently showed major cardiovascular events and death than patients with EPA/AA ratio ≥ 0.35 on statin therapy. In current practice, lipid management as stroke therapy is emphasized. From our data, a combination of baseline EPA/AA ratio and statin therapy could be critical in predicting and optimizing the long-term prognosis of ischemic stroke patients.

In the present study, only 4% of patients received n-3 PUFA formulations after stroke, which might have influenced the prognosis, but no significant difference was identified because of the small sample size. From our data, the efficacy of therapeutic intervention with n-3 PUFA formulations for preventing major cardiovascular events and death among ischemic stroke patients with low EPA/AA remains unknown. On the other hand, our data elucidated that high age, low BMI, and high NIHSS score and D-dimer level were related to major cardiovascular events and death. A previous study showed that age, NIHSS score, and D-dimer were linked to poor outcomes, including stroke recurrence and mortality, and these factors might therefore be the confounding factors in the current investigation. Associations of frailty with both mortality and low n-3 PUFA levels have recently been reported. Future studies focusing on these factors in relation to n-3 PUFA levels among ischemic stroke patients may thus be warranted.

Some potential limitations of this study must be considered when interpreting the present results. First, data from the present study were derived from a single center, and the number of patients with major cardiovascular events and death was quite small. Second, this study used a retrospective design, and 74 patients were lost to follow-up. Treatments after stroke such as statins and n-3 PUFA formulations were not randomized. Third, plasma PUFA levels were examined within 24 h of admission or with the referral to the Department of Neurology in patients who developed ischemic stroke during hospitalization, and the EPA/AA ratio therefore could have been affected by the stroke itself. Fourth, detailed information on dietary habits, daily compliance with medications, and monitoring of EPA/AA ratio was not obtained from the medical records. Collectively, some bias regarding the selection of patients and treatment effects might therefore have been presented in the final analysis, and this represents an issue regarding the generalizability of the present results to the wider population with ischemic stroke.

**Conclusion**

EPA/AA ratio at stroke onset and statin therapy are important to predict long-term prognosis in ischemic stroke patients. Ischemic stroke patients with low EPA/AA ratio at baseline and treatment without statins should be carefully considered in terms of long-term prognosis.
Acknowledgements

None.

Funding

None.

Potential Conflicts of Interest

R.T. received lecture fees from Daiichi Sankyo Co., Boehringer Ingelheim, Otsuka Pharmaceutical Co., Ltd., Takeda Pharmaceutical Co., Ltd., Novartis Pharmaceuticals, Sumitomo Dainippon Pharma Co., Ltd., and Astellas Pharma Inc.

T.U. received lecture fees from Boehringer Ingelheim, AstraZeneca K.K., Bayer Pharmaceutical Co., Ltd., Otsuka Pharmaceutical Co., Ltd., Daiichi Sankyo Co., AbbVie GK., and research funds from Boehringer Ingelheim, AstraZeneca K.K., Otsuka Pharmaceutical Co., Ltd., Astellas Pharma Inc., Eisai Co., Ltd., Bayer Pharmaceutical Co., Ltd., Sumitomo Dainippon Pharma Co., Daiichi Sankyo Co., Ltd., Takeda Pharmaceutical Co., Ltd., Merck Sharp and Dohme (MSD) K.K., AbbVie GK.

N.H. was an advisory member of Dai-Nippon Sumitomo Pharma Co., Ltd., Hisamitsu Pharmaceutical Co., Inc, Biogen Idec Japan Ltd., received lecture fees from Dai-Nippon Sumitomo Pharma Co., Ltd., Otsuka Pharmaceutical Co., Ltd., Takeda Pharmaceutical Co., Ltd., Kyowa Hakko-Kirin Co., Ltd., FP Pharmaceutical Corporation, Eisai Co., Ltd., Novartis Pharma K.K. and AbbVie GK., and received department endowments from the following commercial entities: Kyowa Hakko-Kirin Co., Ltd., Nippon Boehringer Ingelheim, Co., Ltd., AbbVie GK, FP Boehringer Corporation, Otsuka Pharmaceutical, Co., Ltd., Dai-Nippon Sumitomo Pharma Co., Ltd., Eisai Co., Ltd., Nihon Medi-physics Co., Ltd., Asahi Kasei Medical Co., Ltd., Ono Pharmaceutical Co., Ltd., MiZ Co, Ltd., AbbVie GK, OHARA Pharmaceutical Co., Ltd., Nihon Pharmaceutical Co., Ltd., Mitsubishi Tänebe Pharma Corporation, Boston Scientific Corporation, and Medtronic, Inc.

The remaining authors report no conflicts of interest.

References

1) Hankey GJ: Long-term outcome after ischaemic stroke/transient ischaemic attack. Cerebrovasc Dis, 2003; 16 Suppl 1: 14-19

2) Montaner J, Garcia-Berrocoso T, Mendioroz M, Palacios M, Perea-Gainza M, Delgado P, Rosell A, Slevin M, Ribo M, Molina CA, Alvarez-Sabin J: Brain natriuretic peptide is associated with worsening and mortality in acute stroke patients but adds no prognostic value to clinical predictors of outcome. Cerebrovasc Dis, 2012; 34: 240-245

3) Segal HC, Burgess AL, Poole DL, Mehta Z, Silver LE, Rothwell PM: Population-based study of blood biomarkers in prediction of subacute recurrent stroke. Stroke, 2014; 45: 2912-2917

4) He K, Song Y, Daviglus ML, Liu K, Van Horn L, Dyer AR, Goldbourt U, Greenland P: Fish consumption and incidence of stroke: a meta-analysis of cohort studies. Stroke, 2004; 35: 1538-1542

5) Iso H, Kobayashi M, Ishihara J, Sasaki S, Okada K, Kata Y, Kokubo Y, Tsugane S, Group JS: Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-Based (JPHC) Study Cohort I. Circulation, 2006; 113: 195-202

6) Kromhout D, Bosschieter EB, de Lezenne Coulonder C: The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med, 1985; 312: 1205-1209

7) Tänaka K, Ishikawa Y, Yokoyama M, Origasa H, Matsu- zaki M, Saito Y, Matuszewa Y, Sasaki J, Oikawa S, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata K, Shimada K, Shirato K, JELIS Investigators J: Reduction in the recurrence of stroke by eicosapentaenoic acid for hypercholesterolemic patients: subanalysis of the JELIS trial. Stroke, 2008; 39: 2052-2058

8) De Caterina R: n-3 fatty acids in cardiovascular disease. N Engl J Med, 2011; 364: 2439-2450

9) De Caterina R, Cybulsky MI, Clinton SK, Gimbrone MA, Jr., Libby P: The omega-3 fatty acid docosahexaen- ate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. Arterioscler Thromb, 1994; 14: 1829-1836

10) Lavie CJ, Milani RV, Mehra MR, Ventura HO: Omega-3 polyunsaturated fatty acids and cardiovascular diseases. J Am Coll Cardiol, 2009; 54: 585-594

11) Domei T, Yokoi H, Kuramitsu S, Soga Y, Arita T, Ando K, Shirai S, Kondo K, Sakai G, Goya M, Iwabuchi M, Ueda M, Nobuyoshi M: Ratio of serum n-3 to n-6 polyunsaturated fatty acids and the incidence of major adverse cardiac events in patients undergoing percutaneous coronary intervention. Circ J, 2012; 76: 423-429

12) Hishikari K, Kimura S, Yamakami Y, Kojima K, Sagawa Y, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata K, Shimada K, Shirato K: The prognostic value of the serum eicosapentaenoic acid to arachidonic acid ratio in relation to clinical outcomes after endovascular therapy in patients with peripheral artery disease caused by femoropopliteal artery lesions. Atherosclerosis, 2015; 203: 583-588

13) Nozue T, Yamamoto S, Toyama K, Sasaki S, Okada K, Kobayashi M, Ishihara J, Sasaki S, Okada K, Kata Y, Kokubo Y, Tsugane S, Group JS: Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-Based (JPHC) Study Cohort I. Circulation, 2006; 113: 195-202

14) Watanabe S, Yoshihisa A, Kanno Y, Takiguchi M, Yoko- kawa T, Sato A, Miura S, Shimizu T, Abe S, Sato T,
15) Ueno Y, Tanaka R, Yamashiro K, Miyamoto N, Hira K, Kurita N, Sakurai M, Urabe T, Shimada K, Miyazaki T, Daida H, Hattori N: Age Stratification and Impact of Eicosapentaenoic Acid and Docosahexaenoic Acid to Arachidonic Acid Ratios in Ischemic Stroke Patients. J Atheroscler Thromb, 2018; 25: 593-605

16) Adams HP, Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE, 3rd: Classification of sub-type of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke, 1993; 24: 35-41

17) Ueno Y, Yamashiro K, Tanaka R, Kuroki T, Hira K, Kurita N, Urabe T, Hattori N: Emerging Risk Factors for Recurrent Vascular Events in Patients With Embolic Stroke of Undetermined Source. Stroke, 2016; 47: 2714-2721

18) Nishizaki Y, Shimada K, Tani S, Ogawa T, Ando J, Takahashi M, Yamamoto M, Shinozaki T, Miyauchi K, Nagao K, Hirayama A, Yoshimura M, Komuro I, Nagai R, Daida H: Significance of imbalance in the ratio of serum n-3 to n-6 polyunsaturated fatty acids in patients with acute coronary syndrome. Am J Cardiol, 2014; 113: 441-445

19) Aikawa T, Miyazaki T, Shimada K, Sugita Y, Shimizu M, Ouchi S, Kadoguchi T, Yokoyama Y, Shiozawa T, Hiki M, Takahashi S, Al Shahi H, Dohi S, Amano A, Daida H: Low Serum Levels of EPA are Associated with the Size and Growth Rate of Abdominal Aortic Aneurysm. J Atheroscler Thromb, 2017; 24: 912-920

20) Otsuka R, Tange C, Nishita C, Tomida M, Kato Y, Imai T, Ando F, Shimokata H: Fish and Meat Intake, Serum Eicosapentaenoic Acid and Docosahexaenoic Acid Levels, and Mortality in Community-Dwelling Japanese Older Persons. Int J Environ Res Public Health, 2019; 16:

21) Nagata M, Hata J, Hirakawa Y, Mukai N, Yoshida D, Ohara T, Kishimoto H, Kawanoh H, Kitazono T, Kiyohara Y and Ninomiya T: The ratio of serum eicosapentaenoic acid to arachidonic acid and risk of cancer death in a Japanese community: The Hisayama Study. J Epidemiol, 2017; 27: 578-583

22) Suda S, Katsumata T, Okubo S, Kanamaru T, Suzuki K, Watanabe Y, Katsura K, Katayama Y: Low serum n-3 polyunsaturated fatty acid/n-6 polyunsaturated fatty acid ratio predicts neurological deterioration in Japanese patients with acute ischemic stroke. Cerebrovasc Dis, 2013; 36: 388-393

23) Lewis RA, Austen KE, Soberman RJ: Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. N Engl J Med, 1990; 323: 645-655

24) Tagawa H, Shimokawa H, Tagawa T, Kuroiwa-Matsumoto M, Hirooka Y, Takeshita A: Long-term treatment with eicosapentaenoic acid augments both nitric oxide-mediated and non-nitric oxide-mediated endothelium-dependent forearm vasodilatation in patients with coronary artery disease. J Cardiovasc Pharmacol, 1999; 33: 633-640

25) Cawood AL, Ding R, Napper FL, Young RH, Williams JA, Ward MJ, Guimundsen O, Vige R, Payne SP, Ye S, Shearman CP, Gallagher PJ, Grimble RF, Calder PC: Eicosapentaenoic acid (EPA) from highly concentrated n-3 fatty acid ethyl esters is incorporated into advanced atherosclerotic plaques and higher plaque EPA is associated with decreased plaque inflammation and increased stability. Atherosclerosis, 2010; 212: 252-259

26) Amarenco P, Labreuche J: Lipid management in the prevention of stroke: review and updated meta-analysis of statins for stroke prevention. Lancet Neurol, 2009; 8: 453-463

27) Amarenco P, Bogousslavsky J, Callahan A, 3rd, Goldstein LB, Hennerici M, Rudolph AE, Sillesen H, Simunovic L, Szarek M, Welch KM, Zivin JA, Stroke Prevention by Aggressive Reduction in Cholesterol Levels I: High-dose atorvastatin after stroke or transient ischemic attack. N Engl J Med, 2006; 355: 549-559

28) Kernan WN, Ovbiagele B, Black HR, Bravata DM, Chimowitz MI, Ezekowitz MD, Fang MC, Fisher M, Furie KL, Heck DV, Johnston SC, Kasner SE, Kittner SJ, Mitchell PH, Rich MW, Richardson D, Schwamm LH, Wilson JA, American Heart Association Stroke Council CoC, Stroke Nursing CoCC and Council on Peripheral Vascular D: Guidelines for the prevention of stroke in patients with stroke and transient ischemic attack: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke, 2014; 45: 2160-2236

29) Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, Biller J, Brown M, Demaerschalk BM, Hoh B, Jauch EC, Kidwell CS, Leslie-Mazwi TM, Ovbiagele B, Scott PA, Sheth KN, Southerland AM, Summers DV, Tirschwell DL, American Heart Association Association Stroke: 2018 Guidelines for the Early Management of Patients With Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. Stroke, 2018; 49: e46-e110

30) Ueno Y, Yamashiro K, Tanaka Y, Watanabe M, Miyamoto N, Shimada Y, Kuroki T, Tanaka R, Miyauchi K, Daida H, Hattori N, Urabe T: Rosuvastatin may stabilize atherosclerotic aortic plaque: transesophageal echocardiographic study in the ÉPISTEME trial. Atherosclerosis, 2015; 239: 476-482

31) Zhang J, Liu L, Tao J, Song Y, Fan Y, Gou M and Xu J: Prognostic role of early D-dimer level in patients with acute ischemic stroke. PLoS One, 2019; 14: e0211458

32) Denti L, Scoditti U, Tonelli C, Saccavini M, Caminiti C, Valcavi R, Benatti M and Ceda GP: The poor outcome of ischemic stroke in very old people: a cohort study of its determinants. J Am Geriatr Soc, 2010; 58: 12-17

33) Soares I, Abecasis P and Ferro JM: Outcome of first-ever acute ischemic stroke in the elderly. Arch Gerontol Geriatr, 2011; 53: e81-87

34) Kitagawa M, Haji S and Amagai T: Elevated Serum AA/ EPA Ratio as a Predictor of Skeletal Muscle Depletion in Cachexic Patients with Advanced Gastro-intestinal Cancers. In Vivo, 2017; 31: 1003-1009

35) Myint PK, M OB, Loke YK, S DM, Price GM, Hale R, Metcalf AK, Turner DA, Day DJ, E AW and Potter JF: Important factors in predicting mortality outcome from stroke: findings from the Anglia Stroke Clinical Network Evaluation Study. Age Ageing, 2017; 46: 83-90