



Significance of Serum Polyunsaturated Fatty Acid Level Imbalance in Patients with Acute Venous Thromboembolism

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Aim: Polyunsaturated fatty acids (PUFAs) take part in various biological events linked to the pathogenesis of venous thromboembolism (VTE), including inflammation, endothelial dysfunction, and hypercoagulability. Several studies have demonstrated the association between PUFAs and the occurrence of VTE. However, the role of PUFAs in the pathogenesis of VTE remains unclear.

Methods: We enrolled 45 patients with acute VTE and 37 age-, gender-, and body mass index-matched healthy volunteers to examine their PUFA levels. Serum omega 3 (eicosapentaenoic acid: EPA and docosahexaenoic acid: DHA) and omega 6 (dihomogammalinolenic acid: DGLA and arachidonic acid: AA) fatty acids levels were measured within 24 h of admission.

Results: Patients with VTE showed significantly higher AA and lower EPA levels, and lower EPA/AA ratios than the controls. Multivariate analysis revealed that AA was an independent marker for VTE. In addition, we divided the patients based on their median age (58 years old). The younger patients with VTE showed significantly lower EPA/AA levels than their age-matched controls, whereas older patients with VTE showed a significantly higher AA/DGLA levels than the older controls.

Conclusions: High serum AA levels and low EPA levels are associated with the development of acute VTE, suggesting that the imbalance of PUFAs may be a potential therapeutic target for preventing acute VTE.

Key words: Fatty acids, Inflammation, Eicosapentaenoic acid, Dihomogammalinolenic acid, Arachidonic acid

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Introduction

Polyunsaturated fatty acids (PUFAs) are involved in the development of cardiovascular diseases, such as coronary artery disease, congestive heart failure, and arrhythmia¹⁻⁶. In addition, PUFAs are linked to various biological events that lead to the pathogenesis of venous thromboembolism (VTE), including inflammation, endothelial dysfunction, and the onset of hypercoagulable states⁷⁻¹⁴. Dietary intake of fish-derived omega-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has been rec-

ommended to reduce the risk of cardiovascular disease^{1, 15}. Several studies have demonstrated an association between PUFAs and the occurrence of VTE¹⁶⁻¹⁸. However, the roles of PUFAs in the pathogenesis of VTE remain unclear.

The prevalence of VTE in Japan, which is almost one-eighth of that in the US, has increased 2.25-fold over the course of a single decade¹⁹. We have previously reported an imbalance of serum PUFA levels, lower EPA/AA ratio, in young Japanese adults living in urban areas²⁰, suggesting that Westernized dietary habits affect VTE prevalence. The aim of this study is to clarify whether low omega-3 PUFAs and high omega-6 PUFAs are associated with the occurrence of VTE. In addition, we investigated the difference of PUFA levels between younger and older patients with VTE.

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Table 1. Characteristics and polyunsaturated fatty acids levels of study subjects

	Control (n=37)	VTE (n=45)	P
Age (years)	55 ± 11	57 ± 17	NS
Male	17 (46%)	20 (44%)	NS
BMI (kg/m ²)	24.0 ± 2.8	24.8 ± 4.0	NS
Current smoker	6 (16%)	4 (9%)	NS
Hypertension	2 (5%)	11 (24%)	0.019
Hyperlipidemia	3 (8%)	5 (11%)	NS
Diabetes mellitus	2 (5%)	4 (9%)	NS
TC (mg/dL)	217 ± 37	180 ± 53	<0.001
TG median (IQR) (mg/dL)	85 (71, 122)	93 (65, 146)	NS
HDL-C (mg/dL)	70 ± 16	50 ± 17	<0.001
LDL-C (mg/dL)	126 ± 36	107 ± 38	0.033
FPG (mg/dL)	100 ± 14	106 ± 34	NS
HbA1c (%)	5.5 ± 0.9	5.6 ± 0.6	NS
Creatinine (mg/dL)	0.8 ± 0.2	0.8 ± 0.5	NS
CRP, median (IQR) (mg/dL)	0.03 (0.01, 0.08)	1.5 (0.5, 2.7)	<0.001
CRP >0.3 mg/dL	4 (11%)	38 (84%)	<0.001
D-dimer (IQR) (µg/mL)	NA	7.3 (5.2, 16.2)	NA
PUFAs			
EPA (µg/ml)	61.5 ± 32.7	53.1 ± 45.7	0.038
DHA (µg/ml)	109.3 ± 39.4	130.4 ± 52.9	NS
DGLA (µg/ml)	30.6 ± 8.7	34.3 ± 13.7	NS
AA (µg/ml)	139.5 ± 27.7	173.0 ± 65.6	0.003

Continuous variables are shown as means ± standard deviation or medians (inter quartile range [IQR]). VTE=venous thromboembolism, BMI=body mass index, TC=total cholesterol, TG=triglyceride, HDL-C=high-density lipoprotein cholesterol, LDL-C=low-density lipoprotein cholesterol, FPG=fasting plasma glucose, CRP=C reactive protein, PUFAs=polyunsaturated fatty acids, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid, DGLA=dihomogammalinolenic acid, AA=arachidonic acid.

Methods

Study Subjects

We enrolled 45 patients, using consecutive patient sampling, who were admitted to Juntendo Hospital, Japan, from September 2011 to August 2014 with acute VTE. VTE was diagnosed using both contrast enhanced computed tomography (CT) and venous ultrasonography. Patients with malignancy were excluded. We also enrolled 37 age-, gender-, and body mass index (BMI)-matched healthy volunteers from 200 consecutive subjects who underwent a medical check-up at a medical center²⁰⁻²². We compared the PUFA levels between the groups. Furthermore, we investigated the association of PUFA levels with VTE in younger and older population. All subjects gave informed consent, and the study was approved by the local ethical committee.

Blood pressure was measured with a standard mercury sphygmomanometer. Height and weight were measured using an automated scale, and BMI was calculated as the weight in kilograms divided by the square of a patient's height in meters. Hypertension

was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, or currently taking antihypertensive medications. Dyslipidemia was classified as any of the following parameters: low-density lipoprotein cholesterol (LDL-C) level ≥ 140 mg/dl, high-density lipoprotein cholesterol (HDL-C) level < 40 mg/dl or triglycerides (TG) level ≥ 150 mg/dl; it was also assigned if patients were taking lipid-lowering medications for the duration of the study. Diabetes mellitus was defined as a documented history of diabetes treated with medications, a hemoglobin A1c (HbA1c) level of ≥ 6.5% according to National Glycohemoglobin Standardization Program guidelines, a fasting plasma glucose level ≥ 126 mg/dl, or non-fasting plasma glucose level ≥ 200 mg/dl.

Blood Sampling

Whole blood samples were drawn after an overnight fast, within 24 h of admission. Serum omega-3 (EPA and DHA) and omega-6 (dihomogammalinolenic acid: DGLA and arachidonic acid: AA) fatty acids levels were measured by Gas Chromatography–Flame Ionization Detector system, as described previously²⁰.

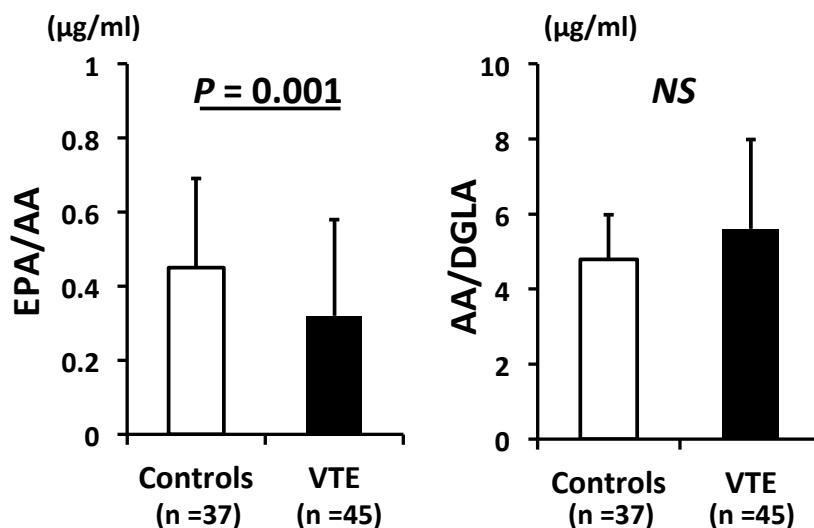


Fig. 1. Comparison of EPA/AA and AA/DGLA ratios between the VTE groups and controls

Patients with VTE showed significantly lower EPA/AA ratios (left panel), and significantly higher AA/DGLA ratios than the controls (right panel).

Serum levels of total cholesterol (TC), TG, and HDL-C were measured by standard enzymatic methods, and LDL-C values were calculated using the Friedewald formula²³). Plasma glucose concentrations, HbA1c, C-reactive protein (CRP), and creatinine levels were measured using standardized methods.

Statistical Analysis

Continuous variables were expressed as mean \pm standard deviation or median (inter quartile range [IQR]), and categorical variables were reported as percentages. Statistical differences between the groups were analyzed by Student's *t*-test, the chi-square test, or the Mann-Whitney-Wilcoxon rank-sum test, as appropriate. The correlations between two parameters were determined by a simple linear regression analysis. Multivariate logistic regression modeling incorporated age, gender, BMI, and factors associated with VTE (hypertension, TC, HDL-C, and CRP >0.3 mg/dL). All statistical analyses were performed using JMP 12 software for Windows (SAS Institute, Cary, NC, USA.). Statistical significance was defined as $P < 0.05$.

Results

The risk factors for VTE varied and included: inherited coagulation disorder (15.6%), prolonged immobilization (15.6%), recent major surgery and/or fracture (13.3%), connective tissue disease and/or steroid use (13.3%), venous aneurysm (2.2%) and no potential risk factor (40%). As shown in **Table 1**,

patients with VTE were more likely to have hypertension, and showed significantly lower levels of TC, HDL-C and LDL-C, and markedly higher levels of CRP than the controls. Compared to the controls, the VTE patients contained a larger number of patients with CRP greater than upper limit of normal (0.3 mg/dL). Serum EPA levels in the VTE group were significantly lower than those in the controls ($P=0.038$), whereas serum AA levels in the VTE group were significantly higher than those in the controls ($P=0.003$). There were no significant differences in serum levels of DHA, and DGLA between the VTE group and controls. As shown in **Fig. 1**, patients with VTE showed significantly lower EPA/AA ratio (indicating a imbalance of omega-3 and omega-6 PUFA) than the controls, whereas AA/DGLA ratio (indicating the increased activity of delta5 fatty acid desaturase²⁴) did not differ between the groups. Multivariate analysis revealed that AA was an independent marker for acute VTE even after adjustment for age, gender, BMI, hypertension, TC, HDL-C, and CRP >0.3 mg/dL, whereas EPA was not an independent marker for acute VTE (**Table 2**).

We have previously reported an extremely lower EPA/AA ratio in younger Japanese adults living in urban areas²⁰). In this study population, increased EPA/AA ratio was also observed in the elderly patients. Therefore, we divided the patients based on their median age (58 years old). The younger patients with VTE showed significantly lower EPA/AA levels than the controls (0.20 ± 0.12 vs. 0.39 ± 0.22 , $P=0.002$),

Table 2. Multivariate logistic regression analysis for the occurrence of venous thromboembolism

	Univariate			Multivariate		
	OR	95% CI	P	OR	95% CI	P
Age	1.01	0.98-1.04	0.51	1.02	0.94-1.14	0.61
Male	0.94	0.39-2.26	0.89	0.41	0.02-5.52	0.49
BMI	1.13	0.99-1.31	0.06	1.11	0.80-1.65	0.54
Hypertension	5.66	1.39-38.3	0.01	1.44	0.02-65.5	0.85
TC	0.98	0.97-0.99	<0.001	0.96	0.92-0.99	0.01
HDL-C	0.93	0.90-0.96	<0.001	0.98	0.91-1.04	0.51
CRP >0.3 mg/dL	44.8	13.3-191.1	<0.001	161.8	13.2-6747	<0.001
EPA	0.99	0.98-1.01	0.34	1.02	0.99-1.04	0.15
AA	1.02	1.01-1.04	<0.001	1.07	1.02-1.14	<0.001

OR=odds ratio, CI=confidence interval, BMI=body mass index, TC=total cholesterol, HDL-C=high density lipoprotein cholesterol, CRP=C-reactive protein, EPA=eicosapentaenoic acid, AA=arachidonic acid.

while the older group of patients with VTE showed significantly higher AA/DGLA levels than the controls (6.3 ± 2.0 vs. 4.6 ± 1.1 , $P=0.003$), as shown in **Fig. 2**.

Discussion

To our knowledge, this is the first study to compare circulating PUFA levels between patients with acute VTE and healthy controls. This study demonstrates that patients with acute VTE have higher serum AA levels, and lower serum EPA levels and EPA/AA ratios than the age-matched, healthy controls. AA levels, but not EPA levels, were considered to be independent markers for the occurrence of acute VTE. In addition, younger patients with acute VTE showed lower EPA/AA ratios than their healthy, age-matched controls, whereas older patients with VTE showed higher AA/DGLA ratios.

High AA levels were observed in the patients with acute VTE. In older patients, AA/DGLA ratios were also associated with VTE. AA accelerates platelet aggregation and inflammation¹¹, and is processed into various proinflammatory and prothrombotic metabolites by the body²⁵, which contributes to the development of VTE. In contrast, DGLA, which is converted to AA by delta5 desaturase²⁵, exerts anti-inflammatory effects; cyclooxygenase metabolizes DGLA to form the bioactive metabolite prostaglandins1. Unlike prostaglandins2 generated from AA, prostaglandins1 possess anti-inflammatory properties²⁵. An increase in DGLA levels relative to those of AA within inflammatory cells may attenuate the biosynthesis of AA metabolites²⁶. These results suggest that high AA levels and AA/DGLA ratios may reflect the proinflammatory state in the patients with acute VTE. However, a cohort study reported that increased delta5 desaturase activity indi-

cated by AA/DGLA ratio was associated with increased omega-3 PUFAs and a reduced coronary heart disease risk²⁴. Therefore, further prospective study is needed to clarify the role of delta5 desaturase in the pathogenesis of VTE.

EPA, which is derived from fish oil, has antithrombotic and anti-inflammatory effects, and improves endothelial function^{7, 8, 10, 12, 13}. Recently, the beneficial effects of EPA on preventing VTE were reported in an animal model⁷. However, the beneficial effects of EPA on the development of VTE remain controversial in clinical settings. Post-operative DVT in Alaskan natives is rare¹⁶, and has been attributed to their traditional diet that is rich in omega-3 PUFAs²⁷. Recently, it has been reported that 12 patients with pulmonary thromboembolism showed lower EPA/AA ratios than patients with other cardiovascular diseases¹⁸. Another study showed that a diet including more plant-based foods and fish, in addition to less red and processed meat was associated with a lower incidence of VTE¹⁷. However, the same study also found that consumption of omega-3 PUFAs itself did not correlate with the incidence of VTE¹⁷. In this study, lower EPA levels and EPA/AA ratios were observed in patients with VTE. Furthermore, lower EPA/AA ratios were associated with the occurrence of VTE in younger, but not older, patients. Previously, we reported extremely lower EPA/AA levels in young Japanese adults²⁰. These results suggest that the effects of low EPA/AA ratios on the development of VTE were limited to younger subjects with lower dietary intake of EPA.

Because this is a small-sample size and case-control study, we could not evaluate cause-and-effect relationships between PUFAs and VTE. We also have no data on the dietary fatty acid intake of our subjects.

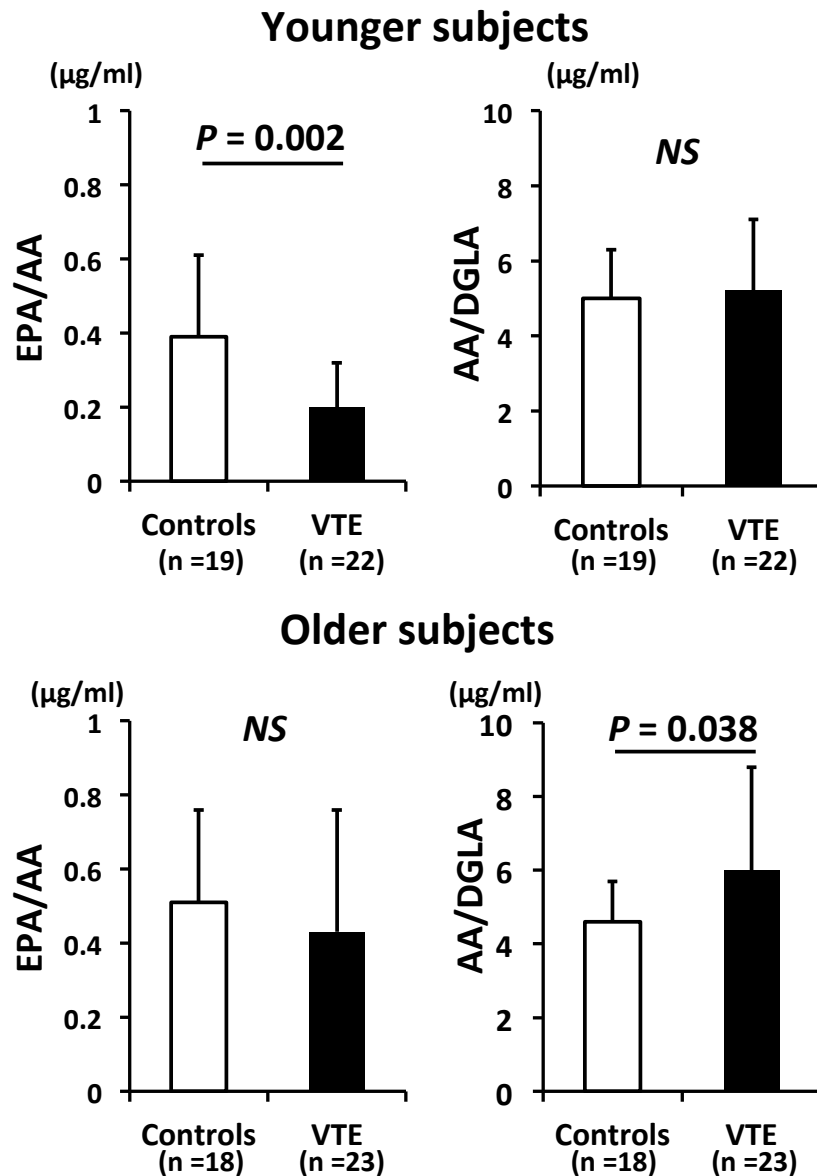


Fig. 2. Comparison of EPA/AA and AA/DGLA ratios between the VTE groups and controls in younger and older subjects

Younger patients with VTE showed significantly lower EPA/AA levels than the controls (left upper panel), whereas AA/DGLA levels did not differ between the controls and VTE group (right upper panel). Older patients with VTE showed no significant difference in EPA/AA levels compared with the controls (left lower panel), whereas AA/DGLA levels in patients with VTE was significantly higher than those in the controls (right lower panel).

Conclusion

High serum AA and low EPA levels are associated with the development of VTE. Additionally, lower EPA/AA levels in younger populations were found to be a risk factor for acute VTE.

Conflict of Interest Disclosure

Dr. Daida has received scholarship funds and lecture fees from Takeda Pharmaceutical Company Ltd. Dr. Miyauchi and Dr. Shimada have also received lecture fees from Mochida Pharmaceutical Company Ltd. and Takeda Pharmaceutical Company Ltd. The remain-

ing authors report no conflicts of interest.

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