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.

See editorial vol. 24: 1011-1013

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

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addition, we investigated the difference of PUFA levels between younger and older patients with VTE.

**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 center. 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 (dihomogammalinole-
nic acid: DGLA and arachidonic acid: AA) fatty acids levels were measured by Gas Chromatography–Flame Ionization Detector system, as described previously. 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 formula. Plasma glucose concentrations, HbA1c, C-reactive protein (CRP), and creatinine levels were measured using standardized methods.

**Statistical Analysis**

Continuous variables were expressed as mean ± 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 an imbalance of omega-3 and omega-6 PUFA) than the controls, whereas AA/DGLA ratio (indicating the increased activity of delta5 fatty acid desaturase) 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 areas. In this study population, increased EPA/AA ratio was also observed in the elderly patients. Therefore, we divided the patients based on their
DGLA ratios may reflect the proinflammatory state in the patients with acute VTE. However, a cohort study reported that increased delta5 desaturase activity indicated by AA/DGLA ratio was associated with increased omega-3 PUFAs and a reduced coronary heart disease risk. 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. Recently, the beneficial effects of EPA on preventing VTE were reported in an animal model. Post-operative DVT in Alaskan natives is rare, and has been attributed to their traditional diet that is rich in omega-3 PUFAs.Recently, it has been reported that 12 patients with pulmonary thromboembolism showed lower EPA/AA ratios than patients with other cardiovascular diseases. However, the same study also found that consumption of omega-3 PUFAs itself did not correlate with the incidence of VTE. 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 adults.

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

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Because this is a small-sample size and case-con-
Younger subjects

![Graph comparing EPA/AA and AA/DGLA ratios between VTE and controls in younger subjects]

- **EPA/AA**
  - Controls (n=19)
  - VTE (n=22)
  - *P = 0.002*

- **AA/DGLA**
  - Controls (n=19)
  - VTE (n=22)
  - *NS*

Older subjects

![Graph comparing EPA/AA and AA/DGLA ratios between VTE and controls in older subjects]

- **EPA/AA**
  - Controls (n=18)
  - VTE (n=23)
  - *NS*

- **AA/DGLA**
  - Controls (n=18)
  - VTE (n=23)
  - *P = 0.038*

**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 lec-
tecture 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 remaining authors report no conflicts of interest.

Acknowledgements
The authors would like to thank Enago (www.enago.jp) for the English language review.

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