

Supplementary Methods

Study population and evaluation

This retrospective cross-sectional study used data from a stroke registry that enrolled patients with acute ischemic stroke.^{1,2} The registry enrolled consecutive patients with acute ischemic stroke or transient ischemic attack within 7 days of symptom onset who were admitted to the Stroke Center of Severance Hospital, Yonsei University, Seoul, South Korea. This study included consecutive patients with nonvalvular atrial fibrillation (AF) and patent foramen ovale (PFO) on transesophageal echocardiography (TEE) between November 2011 and December 2019. Upon admission, all patients underwent brain computed tomography (CT) and/or magnetic resonance imaging with cerebral angiographic studies (CT angiography, magnetic resonance angiography, and digital subtraction angiography); standard blood tests; and chest radiography. Cardiac evaluations included 12-lead electrocardiography (ECG), continuous ECG monitoring during the stay in a stroke unit, 24-hour Holter monitoring, transthoracic echocardiography (TTE), TEE, and heart CT.

Echocardiography evaluation and PFO, atrial thrombus, and spontaneous echo contrast

TTE and TEE were performed within 2 weeks of the initial stroke using commercially available echocardiography machines. TTE was performed using a Vivid E9 ultrasound system (GE Medical Systems, Chicago, IL, USA; Philips iE33; Philips Healthcare, Amsterdam, the Netherlands) with a 2.5–3.5 MHz probe. Standard 2D and Doppler measurements were performed according to the recommendations of the American Society of Echocardiography/European Association of Cardiovascular Imaging.³ The left ventricular ejection fraction (LVEF) was measured using the biplane Simpson's method in the apical four- and two-chamber views. LVEF <52% for males and <54% for females indicated abnormal left ventricular (LV) systolic function.³ In a case of LVEF measured using the M-mode method, the LVEF value minus eight was calculated to substitute LVEF as the biplane method.⁴ Left atrial volume index (LAVI) was measured using the biplane-modified Simpson method in both the apical four- and two-chamber views and indexed to the body surface area. The upper normal limit for 2D echocardiographic LAVI was 34 mL/m² for both sexes.³ If LAVI was measured using the prolate ellipse method, LAVI plus seven was calculated for each patient, and the value was regarded as the LAVI of the Simpson method.⁵ A ratio of early diastolic mitral inflow velocity to early diastolic mitral annular tissue velocity (E/E') >15 indicated an increase in the LV filling pressure.⁶

TEE was performed using either an iE33 xMATRIX ultrasound

system (Philips, Andover, MA, USA) or Acuson SC2000 ultrasound system (Siemens, Mountain View, CA, USA) equipped with a multiplane 5-MHz transducer. TEE was part of the standard and routine evaluation for stroke patients in the study hospital, except for those with decreased consciousness, impending brain herniation, poor systemic condition, and inability to accept an esophageal transducer due to swallowing difficulty, tracheal intubation, or lack of willingness to provide informed consent.^{2,7} If the patients could be trained to perform the Valsalva maneuver, they were properly instructed before the TEE procedure. A contrast test was performed using an agitated saline solution. A PFO was considered present if any microbubbles were seen in the left cardiac chambers within three cardiac cycles of the maximum right atrium enhancement or if a turbulent color jet was seen within the atrial septum on color Doppler images. The physiological shunt size was defined as the maximal number of bubbles observed in the left atrium (LA) either at rest or after the Valsalva maneuver within three cardiac cycles. Shunt size was classified into small (≤ 20 bubbles) and large (> 20 bubbles).^{8,9} Atrial septal aneurysm was defined as ≥ 10 mm of excursion from midline.⁹ All segments of the thoracic aorta, including the ascending and descending aorta and aortic arch, were evaluated for the presence of plaques. The LA appendage (LAA) was observed on the best image between 45° and 90°, including 2D color Doppler images and pulsed-wave Doppler. The LAA emptying velocity (LAAV) was defined as the mean of three consecutive values of peak end-diastolic emptying velocity obtained using pulsed-wave Doppler at the LAA ostium in the TEE view. Spontaneous echo contrast (SEC) was defined as a smoke-like swirling pattern observed in LA or LAA after adjusting for the optimal gain setting.¹⁰ LAAV of > 40 cm/s indicated normal blood flow velocity.¹¹

Echocardiographic parameters, LAAV, LAVI, LVEF, and E/E', which have been suggested to be associated with LA/LAA thrombus and SEC, were assessed.^{12–14} All echocardiographic parameters were used as continuous variables, except in the demographic table.

Statistical analyses

Statistical analyses were performed using R version 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria) and SAS (version 9.4; SAS Institute, Cary, NC, USA). Univariable analysis was performed using the independent t-test, analysis of variance, or Kruskal–Wallis test for continuous variables and the chi-square test for categorical variables, as appropriate. As 17 patients had missing values for the E/E', median imputation was applied to replace the missing values with a median value of 13.

Multivariable logistic regression analyses were performed to determine the association between PFO and atrial thrombus/SEC.

Model 1 was adjusted for age, male sex, and the CHA₂DS₂-VASC score. Model 2 was adjusted for Model 1 factors along with hypertension, diabetes, dyslipidemia, old ischemic stroke, congestive heart failure, ischemic heart disease, current smoking status, and anticoagulation. Model 3 was adjusted using a backward regression analysis. We also performed multivariable logistic regression models considering PFO with and without high-risk features.

To determine potential echocardiographic parameters among LAAV, LAVI, LVEF, and E/E', as a mediator of the relationship between PFO and LA/LAA thrombus or SEC, mediation analysis was performed. This effect was calculated using a comparable coefficient. To perform the mediation analysis, the following pathways were evaluated: (a) the effect of PFO on LAAV; (b) the effect of LAAV on atrial thrombus/SEC; (c) the direct association between PFO and LA/LAA thrombus or SEC (without adjusting for LAAV); and (c') the association between PFO and LA/LAA thrombus or SEC when adjusting for LAAV. Baron and Kenny's proportion of the mediation effect (%) was calculated.¹⁵

Supplementary References

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