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Impaired transport function of the left atrium and left atrial appendage in cryptogenic stroke patients with atrial septal aneurysm and without patent foramen ovale

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Aims Although atrial septal aneurysm (ASA) is frequently combined with patent foramen ovale and associated with cryptogenic stroke (CS), a pathophysiologic correlation between CS and ASA alone has not been fully elucidated. The aims of this study were to assess transport functions of the left atrium (LA) and left atrial appendage (LAA), and to evaluate their relationship in CS subjects with ASA alone.

Methods and results This study consisted of 38 CS subjects with ASA alone and 38 matched controls. Transthoracic echocardiography including tissue Doppler imaging was performed in all subjects and transesophageal echocardiography was conducted in CS subjects to assess LAA emptying velocity (LAAev). We also measured soluble P- and E-selectin, interleukin-6 (IL-6), and high-sensitivity C-reactive protein (hs-CRP) as indices of prothrombogenic and proinflammatory activity. Although there were no differences in left ventricular functions and baseline characteristics between the two groups, CS subjects had significantly larger LA volume and lower LA active pump function compared with controls. LAAev was significantly correlated with LA active function. CS subjects had significantly higher E-selectin ($P = 0.046$), IL-6 ($P = 0.040$), and hs-CRP ($P = 0.001$) compared with controls.

Conclusions Compared with controls, LA active pump function was significantly depressed and closely correlated with LAAev in CS subjects with ASA alone. Moreover, plasma levels of E-selectin, IL-6, and hs-CRP were significantly higher in CS subjects with ASA alone. These findings suggest that impaired LA and LAA functions are a crucial pathophysiologic mechanism for ischaemic stroke in subjects with ASA alone.

Keywords Atrial septal aneurysm • Cryptogenic stroke • Echocardiography • Left atrial function

Introduction

Ischaemic stroke is characterized by a high recurrence rate and significant morbidity and mortality. There are several causes of ischaemic stroke that affect prognosis, outcomes, and management, but in approximately 30–40% of cases of ischaemic stroke the mechanism cannot be determined despite extensive evaluation, such cases are referred to as cryptogenic stroke (CS).[1,2]

Patent foramen ovale (PFO) is more frequently found in CS patients than in healthy adults, and is associated with an increased

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risk of ischaemic stroke.[3–5] Although paradoxical embolism through a PFO has been considered a pathophysiologic mechanism of CS, the role of PFO itself remains unclear. A recent study demonstrated that impaired left atrial (LA) function representing an atrial fibrillation (AF)-like physiology was observed in patients with PFO combined with atrial septal aneurysm (ASA).[6] The authors suggested that LA dysfunction might contribute to LA thrombosis and subsequent ischaemic stroke. Moreover, PFO closure using an Amplatzer Occluder might be beneficial in the improvement of LA function resulting from ASA stabilization. However, little is known about LA and LA appendage (LAA) functions in CS patients with ASA and without PFO.

The aims of this study were to evaluate the LA transport function in CS patients with ASA alone and to assess the association between LA transport function and LAA emptying property. Furthermore, we also compared the plasma levels of biomarker indices of proinflammatory and prothrombogenic activities between CS patients with ASA alone and matched control subjects.

Methods

Study populations

From October 2005 to November 2009, study populations were consecutively selected from 682 patients diagnosed with CS in three Korea institutes (Korea University Guro Hospital, Korea University Ansan Hospital, and Wonkwang University Sanbon Hospital). The diagnosis of CS was established after a systematic aetiologic workup, including brain computed tomography (CT) and/or magnetic resonance (MR) imaging, 12-lead and ambulatory electrocardiograms (ECG), echocardiography, carotid Doppler imaging, cerebral CT or MR angiography, and blood tests. Exclusion criteria included: (i) large artery atherosclerotic stenosis ($\geq$50%) or occlusion of major brain artery or related cortical artery; (ii) lacunar infarcts, defined as a small infarct with a diameter of less than 1.5 cm without any potential extracranial arterial and cardiac sources for embolism; (iii) intracardiac thrombus or any other cause of cardioembolism such as AF, mechanical prosthetic valve, rheumatic mitral stenosis, recent myocardial infarction ($<$4 weeks), dilated cardiomyopathy, akinetic left ventricular (LV) segment, intracardiac tumor, infective endocarditis, spontaneous echocardiographic contrast in the LA, and complex atheroma of the aortic arch; (iv) stroke of other determined aetiology such as non-atherosclerotic arteriopathies, coagulopathies (e.g. the antiphospholipid-antibody syndrome, protein C and S deficiency, etc.); (v) malignancy, hyperthyroidism, uncontrolled hypertension, connective tissue disease, or any acute or chronic inflammatory disease. Individuals without the expected elevation in serologic markers were also excluded (i.e. individuals currently undergoing immunosuppressive therapy or persons with a history of leukopenia of any aetiology). In an attempt to include only CS subjects who had ASA without PFO, transesophageal echocardiography (TEE) was performed in all enrolled subjects. A control group was enrolled, all of a similar age, gender, body mass index (BMI), and atherosclerotic risk factor distributions to the CS subjects. All control subjects were recruited from the Korean Genome Epidemiology Study, an ongoing population-based cohort.[7] The exclusion criteria described above were also applied to controls. Each participant signed an informed consent form prior to enrolling in the study, which was approved by the Human Subjects Review Committee of Korea University and Wonkwang University Hospitals.

Transthoracic echocardiography

All enrolled subjects underwent 2D, M-mode, Doppler, and tissue Doppler imaging (TDI) echocardiography $\geq$3 months after the cerebrovascular episode. All examinations were performed using a commercially available Vivid 7^{TM} (GE Medical System, Vingmed, Horten, Norway) ultrasound system. Three experienced investigator performed the echocardiographic examinations and then all recorded echocardiograms of enrolled subjects were collected to one institute without information about the demographic and clinical characteristics of subjects. Final agreement of the existence of ASA without PFO was achieved by one independent investigator using a computerized off-line analysis station (Echopac^{TM} 6.3.4; GE Medical System).

All measurements were derived from three consecutive cardiac cycles and averaged. For each view, the transducer was carefully angled to quantitatively maximize atrial size, and the gain positions were adjusted to obtain the clearest outline of the endocardium. LV dimensions and wall thicknesses were determined in the parasternal long-axis view with the M-mode cursor positioned just beyond the mitral leaflet tips perpendicular to the long axis of the ventricle according to the recommendations of the American Society of Echocardiography.[8] The LV mass index (LVMI) was determined by the Devereux formula and indexed to the body surface area (BSA).[9] The LV ejection fraction (EF) was obtained via the biplane modified Simpson's method from the apical four- and two-chamber views.

Transmitral pulsed-wave Doppler velocities were recorded from the apical four-chamber view with a 2-mm Doppler sample placed between the tips of the mitral leaflets. Early (E) and late (A) wave velocities and the E/A ratio were assessed from the mitral inflow profile. Pulsed-wave TDI was obtained from the apical four-chamber view. A 2-mm sample volume was placed at the septal and lateral mitral annulus. Systolic (ś), early diastolic (é), and late diastolic (á) velocities were measured and the E/é ratio was subsequently calculated. Diastolic function was categorized as normal, mild or grade I (impaired relaxation), moderate or grade II (pseudonormal LV filling), and severe or grade III (restrictive filling), as previously described and validated.[10]

LA function assessment

To assess the diameter of the LA, the anteroposterior dimension was obtained in the parasternal long-axis view from the leading edge of the posterior aortic wall to the leading edge of the posterior LA wall. To assess the LA volume (LAV), the areas were manually measured after zooming in on the LA by tracing the endocardial border in the apical four- and two-chamber view over the cardiac cycle (*Figure 1*). Special attention was focused on tracing the LA endocardial border. In the apical four-chamber view, if the atrial septum had partially dropped out its location was approximated from visualized fragments. Instead of tracing the inner surface of the mitral valve, a straight line connecting both sides of the mitral leaflet base attachment points to the valve ring was taken as the inferior border of the LA. Both the atrial appendage and the pulmonary veins, when visualized, were carefully excluded at their junction to the LA. The long axis was taken as a line from the midpoint of the mitral valve plane to the superior border of the chamber.[8] The LAV was determined with the biplane area-length (A-L) method and the modified Simpson's method. To assess the LA phasic EF during cardiac cycle, LA volumes were measured at the mitral valve opening (LAV_{max}) at the onset of atrial emptying (LAV_{OAE}, P wave onset of the ECG) and at mitral valve closure (LAV_{min}) from the apical two- and four-chamber views. LAV_{max}, LAV_{OAE}, and LAV_{min} were calculated and indexed to the BSA. Detailed

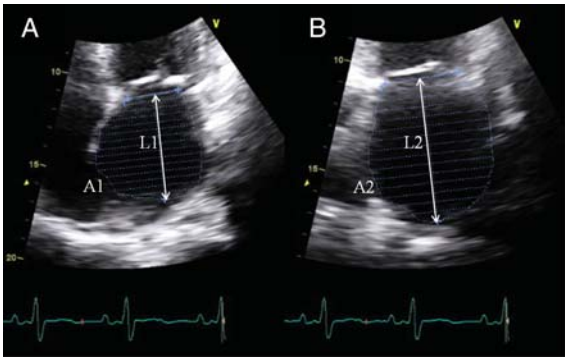

Figure 1 A representative images of the measurement of the left atrial (LA) volume. (*A*) Apical four-chamber view; (*B*) apical two-chamber view. Left atrial areas in the apical four- and two-chamber views (A1 and A2, respectively), and left atrial common long axis (L) can be measured. Left atrial common long axis (L) is the smaller one between L1 and L2. LA volume was calculated using area-length method and modified Simpson's method.

LA transport functions were calculated as follows:

$$\text{LA total emptying fraction } (\text{LAEF}_{\text{total}}) = 100\times\left(\frac{(\text{LAV}_{\max}-\text{LAV}_{\min})}{\text{LAV}_{\max}}\right)$$

$$\text{LA passive emptying fraction } (\text{LAEF}_{\text{passive}}) = 100\times\left(\frac{(\text{LAV}_{\max}-\text{LAV}_{\text{OAE}})}{\text{LAV}_{\max}}\right)$$

$$\text{LA active emptying fraction } (\text{LAEF}_{\text{active}}) = 100\times\left(\frac{(\text{LAV}_{\text{OAE}}-\text{LAV}_{\min})}{\text{LAV}_{\text{OAE}}}\right).$$

The measurements of LA function were performed by two independent investigators. Intra- and inter-observer correlation coefficiency was listed in *Table 1*.

Transesophageal echocardiography

All CS subjects underwent TEE with a Vivid 7^{TM} (GE Medical System) ultrasound system using a 2.9–7.0 MHz multiplane transducer. PFO was diagnosed by the presence of right-to-left passage of contrast bubbles through a valve-like structure either spontaneously or after Valsalva manoeuvre within three cardiac cycles after the complete opacification of the right atrium. ASA was determined to be present when the atrial septum protruded more than 10 mm into either atrium beyond the plane of the septum (*Figure 2A*).[11] The peak left atrial appendage (LAA) emptying flow velocity (LAAev) was measured by placing a pulse Doppler cursor in the LAA about 2 mm from its orifice (*Figure 2B*). The LAAev was determined from three consecutive cardiac cycles and averaged.

Follow-up

All CS subjects were prospectively followed for monitoring of undetected AF for up to 6 months after discharge by monthly outpatient clinic visits. Subjects also underwent 3-channel 48-h Holter monitoring at 1, 3, and 6 months post-discharge. A 12-lead ECG was performed during every visit and anytime when the subjects reported palpitations. If any instances of AF were documented during the 6-month follow-up period, the subject was excluded from the present study.

Blood sampling and analysis

In all CS subjects with ASA alone, a blood sample was obtained from a peripheral vein immediately prior to echocardiographic examinations. Blood samples were drawn into ice-chilled plain tubes containing ethylene diaminetetra-acetic acid, and immediately centrifuged at 3000 rpm (2000 g) at 4°C for 20 min. All samples were stored at −80°C until assayed. Assays of the samples were batched together and were processed by a technician blinded to all subject information. Interleukin-6 (IL-6) and high-sensitivity C-reactive protein (hs-CRP) were measured by enzyme-linked immunosorbent assays (ELISA; Bioo Scientific, Austin, TX, USA and Alpha Diagnostic International, San Antonio, TX, USA, respectively). Serum level of IL-6 was expressed as picogram per millilitre (pg/mL) and hs-CRP level was expressed as milligram per litre (mg/L) and as absolute levels. The RayBio® Human P-Selectin ELISA kit (RayBiotech, Norcross, GA, USA) was used to measure plasma P-selectin. The Human E-Selectin ELISA Kit (R&D System, Abingdon, Oxon, UK) was used to measure plasma E-selectin. Plasma levels of P- and E-selectin were expressed as nanogram per millilitre (ng/mL) and as absolute levels.

Statistical analysis

All continuous variables were expressed as either mean ± standard deviation (SD) or median (25th, 75th interquartile range), depending on distribution. For continuous data, statistical differences were evaluated using the Student's *t*-test or the Mann–Whitney *U* test, depending on data distribution. Categorical variables are presented as frequencies (%), and the chi-square test was used for analysis of categorical variables. All correlations were performed using Spearman's rank correlation test. Bland–Altman analysis was performed to express reproducibility of measurement for ASA excursion between each investigator. All statistical analyses were conducted using SPSS statistical software, version 13.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $P < 0.05$ (two-sided).

Results

Among 682 patients who were diagnosed with CS, 114 subjects (16.7%) had ASA. Out of these 114 subjects, PFO was found in 71 subjects (62.3%) and AF was detected in 5 subjects during a 6-month follow-up. Therefore, this study finally consisted of 38 subjects who had ASA alone and 38 matched controls.

The baseline demographics for both groups are listed in *Table 2*. Blood pressures and glucose levels were well controlled with medications. All CS subjects had been taking anti-thrombotic agents, but there were no statistically differences between the two groups.

Table 3 shows the echocardiographic data for cardiac dimensions, and LV diastolic and systolic functions. All CS subjects with only ASA showed normal echocardiographic studies with similar diastolic/systolic dimensions and functions of the LV compared with matched controls, except for late diastolic velocity (á wave of mitral annulus) of the LV.

Average values of LAVs and transport functions in both groups are presented in *Table 4*. LAVs calculated by the A-L method were larger than those calculated by the modified Simpson's method,

Table 1 Intra- and inter-observer variability in measurements using transthoracic echocardiography (modified Simpson's method)

	Intra-observer variation coefficiency			Inter-observer variation coefficiency		
	r	95% CI	Mean difference	*r*	95% CI	Mean difference
LAV_{max}	0.998	0.994; 1.000	2.85 ± 1.16 mL	0.976	0.988; 0.999	4.03 ± 1.88 mL
LAV_{OAE}	0.997	0.987; 0.999	3.31 ± 2.20 mL	0.997	0.985; 0.999	3.61 ± 3.27 mL
LAV_{min}	0.994	0.975; 0.998	2.71 ± 2.09 mL	0.961	0.965; 0.998	3.04 ± 3.07 mL
$LAEF_{total}$	0.990	0.428; 0.960	1.31 ± 2.61%	0.934	0.958; 0.997	1.50 ± 3.81%
$LAEF_{passive}$	0.981	0.691; 0.982	1.82 ± 2.25%	0.921	0.917; 0.996	2.00 ± 2.69%
$LAEF_{active}$	0.978	0.384; 0.956	1.69 ± 2.05%	0.902	0.689; 0.981	2.56 ± 4.75%

Data are expressed as mean ± SD.
LAV_{max}, maximal left atrial volume; LAV_{OAE}, left atrial volume at the onset of atrial emptying; LAV_{min}, minimal left atrial volume; $LAEF_{total}$, total left atrial emptying fraction; $LAEF_{passive}$, passive left atrial emptying fraction; $LAEF_{active}$, active left atrial emptying fraction.

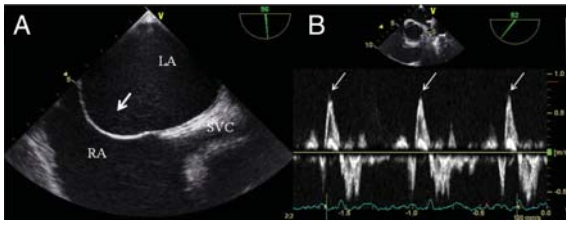

Figure 2 Transesophageal echocardiograms of the cryptogenic stroke patients. (*A*) Atrial septal aneurysm. Protrusion of the atrial septum towards the right atrium is visible (arrow). (*B*) Pulse wave velocities of the left atrial (LA) appendage. The peak LA appendage emptying flow velocities are visualized (arrows). RA, right atrium; SVC, superior vena cava.

but showed an excellent correlation ($r > 0.99$, $P < 0.001$). Although there was no difference in LAV_{OAE} between the groups, LAV_{max} and LAV_{min} were significantly larger in CS subjects with ASA alone compared with controls.

LA reservoir function ($LAEF_{total}$) was smaller in CS subjects with ASA alone than in controls, but the difference was not statistically significant. LA conduit function ($LAEF_{passive}$) was similar between the two groups. LA booster pump function ($LAEF_{active}$) was significantly decreased in CS subjects with only ASA compared with controls. LA booster pump function assessed by TDI (á) in *Table 3* was also significantly lower in CS patients with ASA alone compared with controls. LAEF measured by two different methods showed no significant difference and good correlation ($r > 0.91$). Moreover, intra- and inter-observer variability revealed that the measurements of LAV and LAEF were reproducible and reliable (*Table 1*).

Bland–Altman plot shows the excellent reproducibility of measurement of ASA excursion between each investigator (*Figure 3*).

Figure 4 shows the relationship between LAAev and LA transport functions. LAAev was significantly correlated with $LAEF_{total}$ and $LAEF_{active}$, but there was no relationship between LAAev and $LAEF_{passive}$. Late diastolic velocity (á) of the LV also showed good correlation with LAAev ($r = 0.404$, $P = 0.012$).

Table 2 Baseline characteristics in cryptogenic stroke subjects with atrial septal aneurysm alone and control subjects

Variables	CS group (*n* = 38)	Control group (*n* = 38)	*P* value
Age (years)	55.0 ± 9.5	55.8 ± 10.3	0.730
Male (%)	22 (57.9)	22 (57.9)	1.000
BMI (kg/m^2)	25.6 ± 3.2	25.2 ± 2.3	0.544
Hypertension (%)	18 (47.4)	22 (57.9)	0.358
Diabetes (%)	10 (26.3)	8 (21.1)	0.589
Systolic blood pressure (mmHg)	128.5 ± 11.1	129.4 ± 15.1	0.769
Diastolic blood pressure (mmHg)	80.7 ± 8.7	82.1 ± 9.7	0.519
Heart rate (beats/min)	68.8 ± 10.3	70.7 ± 16.2	0.551
Glucose (mg/dl)	102.7 ± 11.5	101.8 ± 12.4	0.752
Cholesterol, total (mg/dl)	168.2 ± 24.5	165.2 ± 28.9	0.632
Triglyceride (mg/dl)	117 (92–160)	109 (72–187)	0.451
HDL cholesterol (mg/dl)	48.4 ± 12.7	45.3 ± 14.6	0.318
LDL cholesterol (mg/dl)	106.9 ± 19.6	106.4 ± 22.0	0.921
Medications			
ACEi or ARB (%)	15 (39.5)	17 (44.7)	0.642
Beta-blockers (%)	13 (34.2)	16 (42.1)	0.637
CCB (%)	9 (23.7)	10 (26.3)	0.791
Statins (%)	14 (36.8)	17 (44.7)	0.484
Aspirin (%)	22 (57.9)	15 (39.5)	0.108
Clopidogrel (%)	12 (31.6	7 (18.4	0.185
Aspirin + clopidogrel (%)	4 (10.5)	0 (0)	0.115

Data are presented as mean ± SD or median (interquartile range).
BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker.

Table 5 compares plasma levels of biomarker indices for proinflammatory and prothrombogenic activities between CS subjects with ASA alone and controls. Levels of hs-CRP, IL-6, and E-selectin

Table 3 Echocardiographic parameters in cryptogenic stroke subjects with atrial septal aneurysm alone and control subjects

Variables	CS group ($n = 38$)	Control group ($n = 38$)	P value
$LVID_d$ (mm)	49.0 ± 4.3	48.5 ± 4.6	0.586
$LVID_s$ (mm)	29.7 ± 4.1	29.3 ± 4.3	0.651
LVMI (g/m^2)	106.0 ± 20.0	102.3 ± 19.6	0.424
LA diameter (mm)	40.3 ± 6.1	37.9 ± 7.2	0.112
Systolic function			
LVEF (%)	65.2 ± 5.7	64.8 ± 5.4	0.749
ś wave of mitral annulus (cm/s)	7.4 ± 1.0	7.4 ± 1.2	0.772
Diastolic function			
E velocity of mitral inflow (cm/s)	64.0 ± 17.0	61.6 ± 10.9	0.469
A velocity of mitral inflow (cm/s)	64.1 ± 19.9	70.4 ± 20.9	0.182
DT (ms)	215.6 ± 58.5	230.3 ± 53.5	0.257
E/A ratio	1.10 ± 0.5	0.98 ± 0.4	0.236
é velocity of mitral annulus (cm/s)	7.76 ± 2.3	7.20 ± 2.1	0.226
á velocity of mitral annulus (cm/s)*	7.50 ± 1.3	8.52 ± 2.1	0.014
E/ é ratio	8.63 ± 2.8	9.09 ± 2.4	0.441
Diastolic dysfunction (grade)	0.50 ± 0.6	0.68 ± 0.7	0.238
TEE parameter			
LAAev (cm/s)	64.46 ± 18.6	–	–

Data are presented as mean value ± SD.
$LVID_d$, diastolic left ventricular internal dimension; $LVID_s$, systolic left ventricular internal dimension; LVMI, left ventricular mass index; LA, left atrium; LVEF, left ventricular ejection fraction; ś, systolic velocity; E, early velocity; A, late velocity; DT, deceleration time; é, early diastolic velocity; á, late diastolic velocity; TEE, transesophageal echocardiography; LAAev, left atrial appendage peak emptying flow velocity.
*P value < 0.05.

Table 4 LA volumes and functions in cryptogenic stroke subjects with atrial septal aneurysm alone and control subjects

Variables	CS group ($n = 38$)	Control group ($n = 38$)	P value
LA volume (mL)			
LAV_{max} (A-L)*	57.1 ± 18.5	48.7 ± 18.0	0.049
LAV_{OAE} (A-L)	43.4 ± 15.5	37.4 ± 14.1	0.082
LAV_{min} (A-L)*	32.6 ± 11.4	26.3 ± 11.4	0.020
LAV_{max} (S)*	53.1 ± 16.3	44.9 ± 16.8	0.033
LAV_{OAE} (S)	40.6 ± 14.1	34.7 ± 13.1	0.065
LAV_{min} (S)*	30.0 ± 10.4	24.1 ± 10.7	0.017
LA volume index (mL/m^2)			
$LAVI_{max}$ (A-L)	31.8 ± 10.7	27.6 ± 9.6	0.075
$LAVI_{OAE}$ (A-L)	24.2 ± 9.0	21.2 ± 7.6	0.119
$LAVI_{min}$ (A-L)*	18.2 ± 6.7	14.9 ± 6.3	0.031
$LAVI_{max}$ (S)	29.6 ± 9.5	25.4 ± 9.0	0.054
$LAVI_{OAE}$ (S)	22.7 ± 8.2	19.7 ± 7.2	0.099
$LAVI_{min}$ (S)*	16.8 ± 6.2	13.6 ± 5.9	0.025
LA emptying fraction (%)			
$LAEF_{total}$ (A-L)	42.9 ± 9.2	46.5 ± 10.4	0.113
$LAEF_{passive}$ (A-L)	24.2 ± 9.1	23.3 ± 6.7	0.603
$LAEF_{active}$ (A-L)*	24.8 ± 7.5	30.3 ± 11.7	0.016
$LAEF_{total}$ (S)	43.5 ± 8.9	47.3 ± 10.4	0.094
$LAEF_{passive}$ (S)	23.8 ± 8.7	22.6 ± 7.0	0.515
$LAEF_{active}$ (S)*	25.9 ± 7.9	31.8 ± 11.9	0.013

Data are presented as mean value ± SD.
A-L, area-length method; S, modified Simpson's method; LAV_{max}, maximal left atrial volume; LAV_{OAE}, left atrial volume at the onset of atrial emptying; LAV_{min}, minimal left atrial volume; LAVI, left atrial volume index; $LAEF_{total}$, left atrial total emptying fraction; $LAEF_{passive}$, left atrial passive emptying fraction; $LAEF_{active}$, left atrial active emptying fraction.
*P value < 0.05.

were significantly higher in CS patients with ASA alone compared with matched controls.

Discussion

The most noteworthy findings of the present study were: (i) CS patients with ASA alone had larger LAV and lower $LAEF_{active}$ compared with matched controls, even though there were no differences in baseline demographics and other echocardiographic parameters; (ii) LAAev were significantly correlated with LA pump function, especially LA active pump function (á and $LAEF_{active}$); (iii) plasma levels of E-selectin, IL-6, and hs-CRP were significantly higher in CS patients with ASA alone than controls. These findings suggest that functional impairments of LA and LAA might contribute to increased thrombogenicity and a heightened proinflammatory response, ultimately resulting in cardioembolic stroke in patients with ASA alone.

CS is usually defined as stroke with no clearly identifiable cause even after extensive workup and is known to be a benign disease;[12] however, because of its high recurrence rate of up to 30%, many investigators have been striving to determine the currently unknown aetiology. Paradoxical embolism through a PFO is a strong candidate for pathophysiology causing cardioembolic stroke, because the prevalence of PFO among CS patients is about 40%, nearly two-fold higher than in healthy adults.[13] However, the association between cardioembolic stroke and PFO remains controversial.

Several studies have demonstrated that ASA is frequently found in patients with PFO and coexisting ASA and identified PFO as a potential risk factor for recurrent cardioembolic stroke.[11,14,15] The authors hypothesized that *in situ* thrombosis in ASA might be a potential alternative mechanism of cardioembolic stroke. Recently, Rigatelli *et al.*[6] suggested that ASA patients with PFO

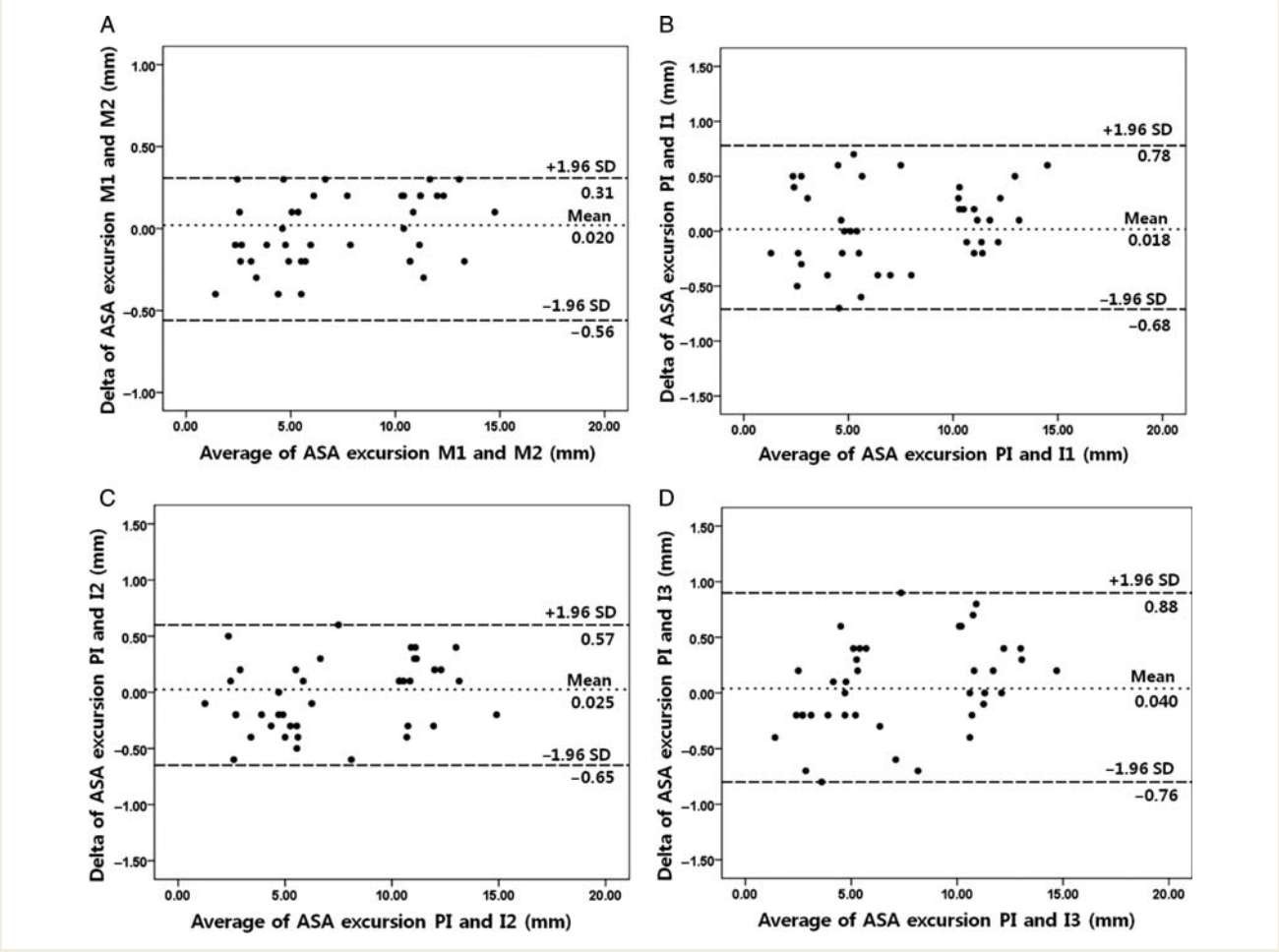

Figure 3 Results of Bland–Altman analysis of agreement for atrial septal aneurysm excursion. (*A*) Result for intra-observer reproducibility of principal investigator (PI). (*B*) Result between PI and investigator 1. (*C*) Result between PI and investigator 2. (*D*) Result between PI and investigator 3. M indicates measurement; PI, principal investigator; I, investigator.

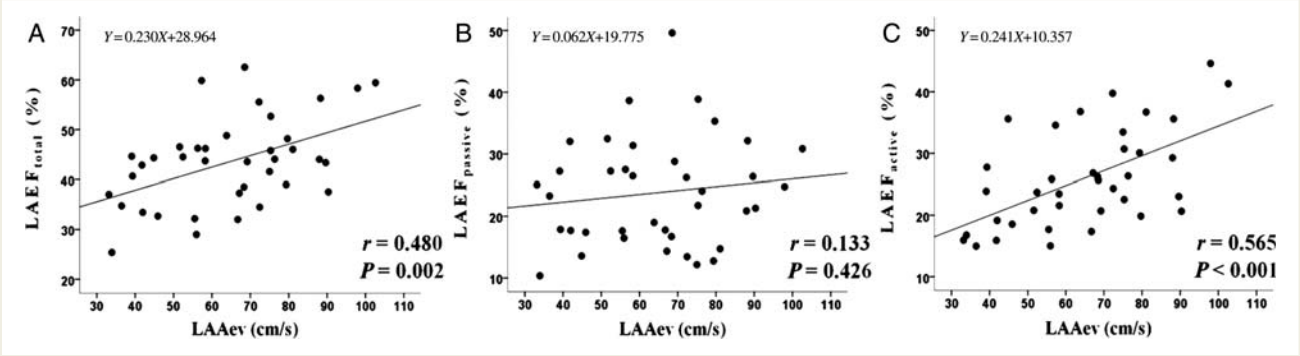

Figure 4 The relationship between left atrial appendage (LAA) emptying flow velocity (LAAev) and left atrial transport functions. $LAEF_{total}$ indicates LA total emptying fraction; $LAEF_{passive}$, LA passive emptying fraction; and $LAEF_{active}$, LA active emptying fraction.

had LA dysfunction, which might contribute to LA thrombosis and subsequent ischaemic stroke. They also demonstrated that PFO closure using an Amplatzer Occluder might be beneficial for improvement of LA functional parameters resulting from ASA stabilization. Moreover, Goch *et al.*[16] revealed that subjects with only ASA had depressed LA systolic function and an enhancement of LAA function, which might be a compensatory mechanism for LA deterioration. Although these findings are in

Table 5 Plasma levels of biomarkers as indices as proinflammatory and prothrombogenic activities in cryptogenic stroke subjects with atrial septal aneurysm alone and control subjects

Variables	CS group ($n = 38$)	Control group ($n = 38$)	*P* value
hs-CRP (mg/L)*	2.40 (1.18–3.80)	1.04 (0.60–1.95)	0.001
IL-6 (pg/mL)*	10.3 ± 3.5	8.4 ± 4.4	0.040
E-selectin (ng/mL)*	55.9 ± 23.7	46.3 ± 17.3	0.046
P-selectin (ng/mL)	67.9 ± 29.9	62.0 ± 33.2	0.424

Data are presented as mean value ± SD or median (interquartile range).
hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6.
*P value < 0.05.

general agreement with the results of the present study, there are several notable differences. In the previous studies, most of enrolled subjects were individuals in the general populations. Therefore, their results cannot represent the underlying pathophysiologic mechanism of ischaemic event in CS patients, because PFO and/or ASA are not independently associated with cerebrovascular events in the general population.[17,18] Furthermore, to exclude any possible aetiology of paradoxical embolism, our study included only CS subjects who had ASA without PFO, as confirmed by TEE. Secondly, in contrast to the results of Goch *et al.*, [16] in our study the emptying function in LAA was depressed in CS patients with ASA alone and closely correlated with LA active pump function. We contend that, in subjects with ischaemic stroke, the pathophysiology of LAA and LA may be quite different from that of the general population. In addition, simultaneous depression of the emptying function in the LA and LAA, which is a major site of thrombus formation,[19] might contribute to an enhanced thrombogenicity in CS patients with ASA alone. Finally, a study from Rodes-Cabau *et al.*[20] showed that brachial flow-mediated dilatation, an index of endothelial function, was significantly lower in CS patients without PFO compared with CS patients with PFO. They suggested that an atherosclerotic-mediated mechanism may be involved in ischaemic stroke in CS patients without PFO. Our data may support this finding since plasma levels of E-selectin, IL-6, and hs-CPR, biomarker indices of prothrombogenic and proinflammatory activity were significantly higher in CS patients with ASA and without PFO than in matched controls. However, their study did not assess plasma biomarkers in CS patients who had only ASA without PFO compared with controls.

Limitations

First, to exclude undetected paroxysmal AF (PAF), we performed standard 12-lead ECGs at every visit and anytime when the subjects reported palpitations, and three periods of 48-h Holter monitoring in all subjects. However, we cannot fully exclude PAF episodes because they are often asymptomatic. Therefore, undetected episodes of PAF may contribute to confounding effects in analyses of LA or LAA function. Second, the effect of various drugs—specifically anti-thrombotic drugs, angiotensin converting enzyme inhibitors or angiotensin receptor blockers, and statins—on LA functions or plasma biomarkers could not be fully controlled for in the present study, as all subjects were not on the same drug therapy regimen. However, there were no significant differences in medication regimens between the two groups. Third, we cannot fully exclude the existence of right-to-left shunt because diagnosis of PFO was only confirmed by TEE using contrast bubbles. Recently, transcranial Doppler examination has been recognized to be more sensitive than TEE in diagnosis right-to-left shunt. Nevertheless, our hypothesis that ASA itself may contribute to thrombosis *in situ* through an LA dysfunction is still plausible. Finally, it is inconclusive whether ischaemic stroke itself triggers inflammatory response or whether LA and LAA dysfunction leads to prothrombogenic or proinflammatory processes in CS subjects with ASA alone. Therefore, further interventional studies are warranted to investigate whether the improvements in LA and/or LAA functions through ASA stabilization would reverse prothrombogenic and/or proinflammatory states.

Conclusions

Compared with matched controls, LA active pump function was substantially depressed in CS patients with ASA alone and closely correlated with the emptying function of LAA. Moreover, plasma levels of E-selectin, IL-6, and hs-CPR, biomarker indices of prothrombogenic and proinflammatory activities, were significantly higher in CS patients who had ASA without PFO. These findings suggest that impaired LA and LAA functions are crucial pathophysiologic factors for a cardioembolic source in CS subjects with ASA alone. Therefore, we infer that life-long anticoagulation is needed in patients with ASA, especially those with prior history of ischaemic stroke.

Conflict of interest: none declared.

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