● さいごに

われわれは，これらアスピリン抵抗性に関する問題，実態を明確にするために，多施設共同前向きコホート研究「アスピリン抵抗性の実態ならびにその遺伝子背景に関する研究」(The Study on Profile and Genetic factors of Aspirin Resistance：ProGEAR Study) を，全国 20 施設の協力のもとに開始した[17]。この試験は，脳梗塞/TIA および急性冠症候群の二次予防としてアスピリンの投与を受けている長期服薬患者 1000 症例の登録を目標に，3 種類の血小板機能検査，2 種類の COX-1 機能測定を測定項目とし，登録後 2 年間の血栓塞栓症の発症を追跡する試験である。同時に，その遺伝子背景を COX-1，COX-2 遺伝子を中心として解析する予定である。

本邦でのアスピリン抵抗性の実態ならびにその背景因子について明らかにできるものと期待している。

この論文の成果の一部は，平成 17 年度厚生労働科学研究費補助金 (循環器疾患等総合研究事業)「抗凝固薬・抗血小板薬の標的およびこれら薬剤を修飾するタンパク質・遺伝子の解析を通した最適投与量の評価方法の標準化に関する研究」による。

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Right-to-Left Shunt Evaluated at the Aortic Arch by Contrast-Enhanced Transesophageal Echocardiography

Masahiro Yasaka, MD, Koichi Ikeno, MD, Ryoichi Otsubo, MD, Hiroshi Oe, MD, Keiko Nagano, MD, Kazuo Minematsu, MD

Objective. The right-to-left shunt (RLS) is diagnosed by contrast-enhanced transesophageal echocardiographic monitoring of the bilateral atria (cTEE-BA). However, the procedure is often disturbed by nonsmoke spontaneous individual contrast (NSSIC) with fast motion, which appears in the left atrium after respiratory maneuvers without administration of a contrast medium and moves past in several seconds. We attempted to perform cTEE monitoring of the aortic arch (cTEE-AA) for evaluation of the RLS and compared the findings with those of cTEE-BA. ***Methods.*** Both cTEE-BA and cTEE-AA were performed in 168 patients with ischemic stroke (133 men and 35 women; mean age ± SD, 62.0 ± 14.4 years). The frequency of NSSIC in the left atrium was compared with that in the aortic arch during the respiratory maneuver. When contrast much brighter than the NSSIC was visualized in the left atrium and the aortic arch during the respiratory maneuver with administration of the contrast medium, we considered the RLS to be positive in the cTEE-BA and cTEE-AA, respectively. Findings were then compared between the 2 examinations. ***Results.*** Nonsmoke spontaneous individual contrast was more frequently observed in the left atrium than the aortic arch (61.3% versus 14.9%; χ^2 test, $P < .0001$). The RLS was positive in 34 patients in the cTEE-BA and in 39 patients in the cTEE-AA. The sensitivity and specificity of the cTEE-AA for the cTEE-BA were 100% and 97.0%, respectively. ***Conclusions.*** The cTEE-AA may be an alternative method for detection of an RLS, especially in patients with a large amount of NSSIC in the left atrium. ***Key words:*** aortic arch; patent foramen ovale; respiratory maneuver; right-to-left shunt; transesophageal echocardiography.

Abbreviations
cTEE-AA, contrast-enhanced transesophageal echocardiographic monitoring of the aortic arch; cTEE-BA, contrast-enhanced transesophageal echocardiographic monitoring of the bilateral atria; NSSIC, nonsmoke spontaneous individual contrast; PAVF, pulmonary arteriovenous fistula; PFO, patent foramen ovale; RLS, right-to-left shunt

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The right-to-left shunt (RLS) represented by the patent foramen ovale (PFO) and pulmonary arteriovenous fistula (PAVF) can be a cause of paradoxical embolism. The PFO is found in about 30% of autopsies.[1] The prevalence of the PFO in patients with stroke is higher than in healthy individuals, and the PFO is more frequently detected in cryptogenic stroke than in stroke of known etiology.[2-4]

Contrast-enhanced transesophageal echocardiographic monitoring of the bilateral atria (cTEE-BA) has enabled us to detect the PFO with a higher degree of sensitivity, contributing to the diagnosis of paradoxical brain embolism.[5] However, in the cTEE-BA examination, respiratory maneuvers frequently induce the transient appearance of mild to moderate contrast in the left atrium, that is, nonsmoke spontaneous individual contrast (NSSIC), independently of venous injections of contrast medium.[6] The NSSIC may lead to false-positive or -negative findings in

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the diagnosis of the RLS. The NSSIC is thought to be rouleaux formation caused by blood flow stasis at the pulmonary vein at the respiratory maneuver.[6]

Conversely, the RLS can be evaluated by cTEE monitoring of the aortic arch (cTEE-AA). We previously reported in 2001 that, after release of the respiratory maneuver, flow of a contrast medium into the left atrium through the PFO could be clearly visualized at the aortic arch.[7] Thereafter, when cTEE has been required, we have performed both cTEE-BA and cTEE-AA routinely. In the current study, we retrospectively investigated the sensitivity and specificity of cTEE-AA for cTEE-BA and compared the frequency of NSSIC between the left atrium and the aortic arch.

Materials and Methods

From October 2001 to September 2002, we performed both cTEE-BA and cTEE-AA in 168 patients with ischemic stroke of unknown origin (133 men and 35 women; mean age ± SD, 62.0 ± 14.4 years).

We performed cTEE using a commercially available real-time 2-dimensional echocardiography system (SSD-2200; Aloka Co, Ltd, Tokyo, Japan) equipped with a 5.0-MHz (variable from 3.5 to 7.5 MHz) phased array omniplane transesophageal transducer after informed consent was obtained from the patient or the patient's family. The frequency of probe transmission and depth of the focal zone were changed according to the depths of the objectives. First, we investigated the left atrium and the aortic arch for smokelike swirling contrast echo at rest, which was observed independently of the respiratory maneuver. Then, without any contrast medium, we inspected the left atrium and the aortic arch NSSIC, which came from the upper part of the left atrium, not from the interatrial septum, and was visualized as individual intense contrast with fast motion after the release of the Valsalva maneuver. The NSSIC had a weaker echo density than microbubbles in the right atrium after contrast medium injection. Then, without any contrast medium, we inspected the left atrium and the aortic arch NSSIC. Subsequently, the contrast medium, a mixture of 9 mL of saline and 1 mL of air, was infused into the right antecubital vein during the Valsalva maneuver. When the right atrium was opacified by the contrast medium, as shown on the monitor, we asked the patient to release the Valsalva maneuver in the cTEE-BA. In the cTEE-AA examination, we asked the patient to release the Valsalva maneuver 3 or 4 seconds after the contrast medium was administered. We regarded individual contrast in the left atrium as coming from the interatrial septum and as bright as the contrast medium in the right atrium as contrast medium in the left atrium. We also regarded individual contrast in the aortic arch that was much brighter than the NSSIC as contrast medium in the aortic arch. When the contrast medium was found in the left atrium within 3 cardiac cycles after release of the Valsalva maneuver, we diagnosed PFO. When contrast medium was found in the left atrium after 3 cardiac cycles, we suspected the presence of PAVF,[5–8] and when found in the aortic arch within 10 cardiac cycles after the release of the Valsalva maneuver, we diagnosed RLS. Assessment of the smokelike echo, the NSSIC, and contrast medium in the left atrium or the aortic arch was performed by agreement of 3 physicians operating the echo machine and probe and injecting the contrast medium. When the PFO in the cTEE-BA or the RLS in the cTEE-AA was positive, we measured the size of the PFO and categorized it into less than 2, 2 to 10, and greater than 10 mm.

We compared the frequencies of the smokelike echo at rest, the NSSIC during the respiratory maneuver without administration of the contrast medium, and the contrast medium during the respiratory maneuver with administration of the contrast medium in the left atrium with those in the aortic arch. We also compared the age, sex, and presence of atrial fibrillation between patients with PFO or PAVF as shown by cTEE-BA and those without and between patients with RLS as shown by cTEE-AA and those without.

Continuous data were expressed as mean ± SD. We used the χ^2 test for analysis of discrete variables and the unpaired t test for analysis of continuous variables. In cases of low cell counts (<5), the Fisher exact test was used instead of the χ^2 test.

Results

Nonsmoke spontaneous individual contrast was more frequently noted in the left atrium than in the aortic arch (61.3% versus 14.9%; χ^2 test, $P < .0001$; Figures 1–3 and Table 1), although a significant difference in the frequency of the smokelike echo and contrast medium was not shown

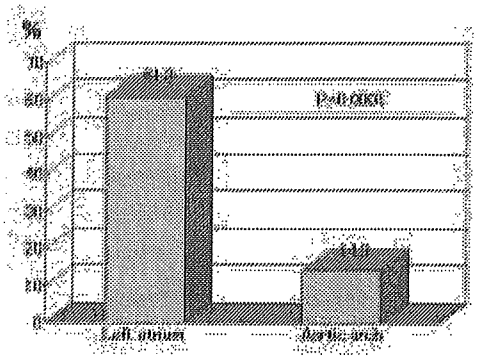

Figure 1. Frequency of NSSIC in the left atrium and the aortic arch.

between the left atrium and the aortic arch. The NSSIC in the aortic arch was frequently accompanied by that in the left atrium (20 [83.3%] of 24).

Patients with NSSIC in the left atrium were younger than those without but not significantly ($P = .12$; Figure 2 and Table 2). The frequency of men was higher in patients with NSSIC in the left atrium than in those without (84.8% versus 69.8%; $P = .0336$). No differences in the frequency of atrial fibrillation and smokelike echo in the left atrium and the aortic arch were observed between patients with NSSIC and those without.

Patients with NSSIC in the aortic arch were significantly younger than those without ($P = .0030$; Figure 3 and Table 3). Men were more prevalent among patients with NSSIC in the aortic arch than those without (87.5% versus 77.8%; $P = .028$), but this difference was not significant. No differences in the frequency of atrial fibrillation and smokelike echo in the left atrium and the aortic arch were observed between patients with NSSIC in the aortic arch and those without.

In cTEE-BA, RLS was shown in 34 patients; PFO was shown in 30 patients; and PAVF was shown in the other 4. In the 30 patients with the diagnosis of PFO, the size of the PFO was less than 2.0 mm in all.

In cTEE-AA, 39 patients had RLS-positive findings. A comparison of the results of cTEE-AA with those of cTEE-BA revealed 2 positive findings in 34 patients and 2 negative findings in 129. The other 5 patients had RLS-positive findings in cTEE-AA but negative findings in cTEE-BA. Of these 5 patients, NSSIC in the left atrium and the aortic arch was positive in both evaluations in 1 patient, positive and negative in 2 patients, negative and positive in 1 patient, and negative in both in 1 patient.

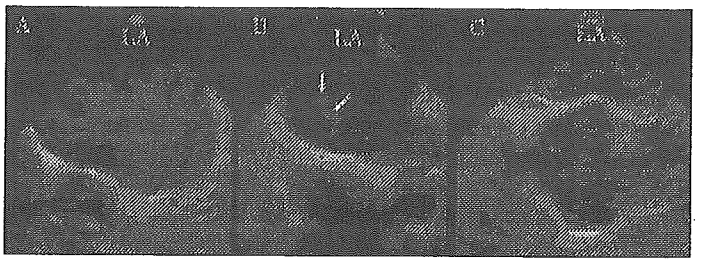

Figure 2. Contrast in the left atrium (LA). **A**, Smokelike echo in the left atrium. **B**, Typical NSSIC in the left atrium intensified by a respiratory maneuver. Arrows indicate NSSIC. **C**, Contrast medium appearing in the left atrium during the respiratory maneuver.

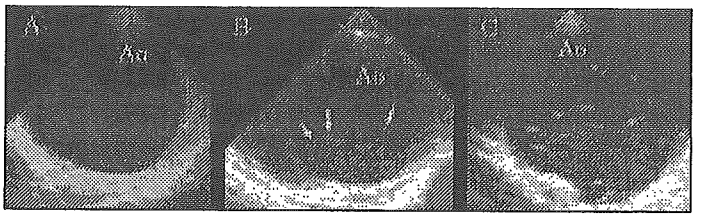

Figure 3. Contrast in the aortic arch (Ao). **A**, Smokelike echo in the aortic arch. **B**, Typical NSSIC in the aortic arch intensified by a respiratory maneuver. Arrows indicates NSSIC. **C**, Contrast medium appearing in the aortic arch during the respiratory maneuver.

The sensitivity, specificity, and accuracy of cTEE-AA for cTEE-BA were 100%, 96.3%, and 97.0%, respectively (Table 4).

Discussion

The NSSIC that appears in the left atrium transiently with fast echo motion by the respiratory maneuver is clearly different from the spontaneous swirling smoke contrast and is called the snowstorm aspect, multiple scattered echoes, or contrast with fast motion.[6,8,9] Although the echo density of the NSSIC is lower than that of the contrast medium appearing in the left atrium in the cTEE-BA examination in patients with PFO, the NSSIC may be a cause of pseudopositive diagnoses for RLS.[6,9] Therefore, it was recommended that a respiratory maneuver should always be performed before contrast medium injections to allow better distinction between NSSIC and true RLS.[6] However, accurate distinction may still be difficult in patients with a small amount of shunting and a large amount of NSSIC or with poor imaging of the bilateral atria.

Table 1. Frequency of the Smokelike Echo, NSSIC, and Contrast Medium in the Left Atrium and the Aortic Arch

Finding	Condition: At Rest	Condition: Respiratory Maneuver	Condition: Administration of Contrast Medium	Frequency, n (%): Left Atrium	Frequency, n (%): Aortic Arch	*P*
Smokelike echo	Yes	No	No	26 (15.5)	38 (22.6)	.0955
NSSIC	No	Yes	No	103 (61.3)	25 (14.9)	<.0001
Contrast medium	No	Yes	Yes	35 (20.8)	39 (23.2)	.6014

Conversely, this current study showed that cTEE-AA had high sensitivity and specificity in diagnosing RLS. The frequency of NSSIC appearing in the aorta was much lower than that in the left atrium, and most patients with NSSIC in the aortic arch had NSSIC in the left atrium as well. This result may be consistent with the cause of NSSIC in the left atrium. Van Camp et al[6] reported that the NSSIC they observed in the left atrium came from the pulmonary vein and disappeared within 3 or 4 cardiac cycles. They then surmised that the NSSIC in the left atrium represented rouleaux formation of red blood cells in the pulmonary vein during the respiratory maneuver, which caused abrupt stasis of blood in the pulmonary vein, and found that the NSSIC disappeared in the bloodstream after release of the respiratory maneuver. This may explain why, in the present study, the frequency of NSSIC in the aortic arch was significantly lower than that in the left atrium, why the NSSIC in the left atrium was common in patients with NSSIC in the aortic arch, and why NSSIC in the left atrium and the aortic arch was related to men and younger age (a stronger respiratory maneuver resulted in greater rouleaux formation).

Five cases had RLS-positive findings by cTEE-AA but negative findings by cTEE-BA. This inconsistency may have been the result of underestimation of cTEE-BA. The NSSIC found in the left atrium in 3 of these cases (60%) may have concealed the true RLS. A small amount of contrast coming through the PFO or PAVF might not have been noticed because it might not have gone through the cross section of the left atrium set by the cTEE-BA. Conversely, the inconsistency may have been due to overestimation of cTEE-AA. The NSSIC in the aortic arch could have been confused with the true contrast of the RLS. However, the detection rate of the true contrast in the cross section of the aortic arch monitored by cTEE-AA would logically be higher than that monitored by cTEE-BA because most particles of the contrast medium pass the aortic arch cross section perpendicular to the bloodstream, except those flowing into the branches of the aortic arch. All the particles of the contrast medium in the left atrium do not go through the cross section of cTEE-BA because it is not always placed perpendicularly to the blood flow in the left atrium.

Table 2. Nonsmoke Spontaneous Individual Contrast in the Left Atrium and Demographics

Demographic	NSSIC: Positive (n = 103)	NSSIC: Negative (n = 65)	*P*
Age, y, mean ± SD	60.6 ± 13.6	64.2 ± 15.0	.12
Men, n (5)	89 (84.8)	44 (69.8)	.036
Atrial fibrillation, n (%)	12 (11.4)	6 (9.5)	.62
Smokelike echo in left atrium, n (%)	17 (16.2)	9 (14.3)	.64
Smokelike echo in aortic arch, n (%)	26 (24.8)	12 (19.0)	.31

Table 3. Nonsmoke Spontaneous Individual Contrast in the Aortic Arch and Demographics

Demographic	NSSIC: Positive (n = 24)	NSSIC: Negative (n = 144)	*P*
Age, y, mean ± SD	54.0 ± 13.5	63.3 ± 14.1	.0029
Men, n (5)	21 (87.5)	112 (77.8)	.28
Atrial fibrillation, n (%)	3 (12.5)	15 (10.4)	.76
Smokelike echo in left atrium, n (%)	2 (8.3)	24 (16.7)	.30
Smokelike echo in aortic arch, n (%)	4 (16.7)	34 (23.6)	.45

Table 4. Findings of cTEE-BA and cTEE-AA

cTEE-AA	cTEE-BA: Positive	cTEE-BA: Negative	cTEE-BA: Total
Positive	34	0	34
Negative	5	129	134
Total	39	129	168

When compared with cTEE-BA, cTEE-AA is not as useful in distinction of PFO from PAVF, which can be differentiated by cTEE-BA. This current study was a retrospective study; thus, a prospective study is required to determine the algorithm that will effectively show RLS, PFO, and PAVF.

In conclusion, because the sensitivity, specificity, and accuracy of cTEE-AA for cTEE-BA are very high, cTEE-AA may be an alternative technique with which to evaluate RLS.

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□ ORIGINAL ARTICLE □

Is Stroke a Paradoxical Embolism in Patients with Patent Foramen Ovale?

Masahiro YASAKA, Ryoichi OTSUBO, Hiroshi OE and Kazuo MINEMATSU

Abstract

Objective Purpose was to assess the stroke mechanism in patients with patent foramen ovale (PFO).

Methods We reviewed the medical records of 111 stroke patients with PFO and sinus rhythm (PFO-S group), 25 with PFO and atrial fibrillation (AF) (PFO-AF group) and 67 with AF but not PFO (AF group), who had received contrast transesophageal echocardiography. The clinical and neuroradiological findings were then compared among the three groups. Deep vein thrombosis was investigated in 93 patients with PFO. We determined the number of patients with definite paradoxical embolism who met three criteria: deep vein thrombosis, neuroradiological features indicating embolic stroke, and the absence of other sources of emboli. We also evaluated those with probable paradoxical embolism who met two of the three criteria.

Results The PFO-S group more frequently exhibited hypercholesterolemia ($p<0.0001$) and lesions limited to the posterior circulation ($p<0.0004$), and less frequently exhibited large or cortical lesions in the anterior circulation ($p=0.0008$, $p<0.0001$, respectively), than the PFO-AF and AF groups. In the PFO-S and PFO-AF groups, other sources of emboli such as a cardiac source of emboli, cerebral artery stenosis ≥50%, or complicated atheroma in the aortic arch were identified in 72 cases (52.9%). In the 93 patients with examination for deep vein thrombosis, the definite and probable criteria of paradoxical embolism were fulfilled only in three (3.2%) and 33 cases (35.5%), respectively.

Conclusion In stroke patients with PFO, not only paradoxical brain embolism through the PFO but also other causes of stroke may contribute to the development of stroke.

(Internal Medicine 44: 434–438, 2005)

Key words: stroke, patent foramen ovale, atrial fibrillation, paradoxical brain embolism

Introduction

Patent foramen ovale (PFO) is found in about 30% of autopsies and may be associated with paradoxical brain embolism (1). The prevalence of PFO in patients with stroke is higher than in control subjects and PFO is more frequently detected in cryptogenic stroke than in stroke of known etiology (2–4). Contrast transesophageal echocardiography (TEE) enables the detection of PFO with a higher degree of sensitivity, which has contributed to the diagnosis of paradoxical brain embolism (5).

Typical patients with paradoxical brain embolism through PFO demonstrate a venous thrombus as the direct source of emboli and neuroradiological features of cerebral embolism. However, numerous stroke patients with PFO do not have a venous thrombus or neuroradiological findings of brain embolism. Therefore, the contribution of paradoxical embolism through PFO to the development of stroke may be smaller than previously thought. Although clarifying the causes of stroke in patients with PFO is important, the clinical characteristics of stroke patients with PFO have not been fully elucidated. Thus, we retrospectively reviewed the medical records of stroke patients having PFO with or without atrial fibrillation (AF) and those of stroke patients with AF but not PFO, and compared their clinical and neuroradiological findings. In addition, we proposed definite and probable criteria for paradoxical brain embolism and determined how many stroke patients with PFO met the criteria.

For editorial comment, see p 401.

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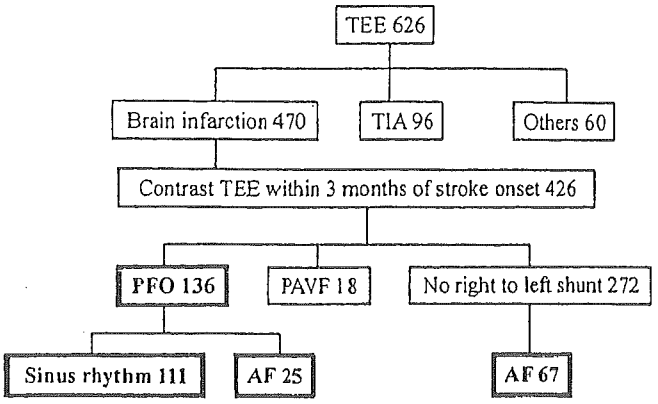

Figure 1. Diagram of the study subjects. TEE: transesophageal echocardiography, TIA: transient ischemic attack, PFO: patent foramen ovale, PAVF: pulmonary arteriovenous fistula, AF: atrial fibrillation. Arabic numerals indicate number of patients.

Methods

From January 2000 to December 2001, we performed TEE in 626 patients (Fig. 1); 470 with brain infarction, 96 with TIA, and the remaining 60 with other neurological disorders. In 426 of the 470 patients with brain infarction, contrast TEE was performed within 3 months of onset. PFO was demonstrated in 136 patients and pulmonary arteriovenous fistula was suspected in 18. We divided the 136 patients with PFO into 111 patients with sinus rhythm (PFO-S group) and 25 patients with AF (PFO-AF group). No right to left shunt was observed in the other 272 patients, and of these patients, AF was noted in 67 patients (AF group) at the time of TEE. We compared the clinical background, atherosclerotic risk factors, vascular territory of the brain infarction, site and size of the infarct, and cerebral angiographic findings among the PFO-S, PFO-AF and AF groups.

We performed contrast TEE using a commercially available real-time two-dimensional echocardiography system (model SSD-2200, Aloka, Tokyo) equipped with a 5.0 MHz phased array omniplane transesophageal transducer. Without any contrast medium, we inspected the left atrium for debris appearing inside the left atrium during the Valsalva maneuver and after release of the maneuver. Next, the contrast medium, a mixture of 9 ml saline and 1ml air, was infused into the right antecubital vein during the Valsalva maneuver. When the right atrium was opacified by the contrast medium as seen on the monitor, we asked the patient to release the Valsalva maneuver. When contrast medium different from the debris was found in the left atrium within three cardiac cycles after the release of the Valsalva maneuver, we diagnosed the patient with PFO, and when the medium was observed after three cardiac cycles, we suspected the presence of pulmonary atriovenous fistula (5–8).

Previously diagnosed hypertension, diabetes mellitus and hypercholesterolemia were considered atherosclerotic risk factors. Patients taking antihypertensive medicine and with a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg were considered hypertensive, while diabetic patients were defined as those taking insulin or oral antidiabetic agents, and exhibiting a fasting plasma glucose level of ≥126 mg/dl or plasma glucose at any time of ≥200 mg/dl. Patients taking antihypercholesterolemic medicine, or with a plasma total cholesterol of ≥220 mg/dl were defined as having hypercholesterolemia.

We investigated the circulation territory, either anterior or posterior circulation, responsible for the infarction, whether the lesions involved cortical areas, and whether the lesions were larger than 3.0 cm in diameter in patients with infarctions of the anterior circulation (9–11).

We also compared the incidence of cervical or cerebral artery stenosis ≥50% demonstrated by carotid ultrasonography, MRA, or cerebral angiography, in the major artery proximal to the responsible infarction, intraluminal filling defect indicating an embolus on angiogram, reopening of the previously occluded artery confirmed by MRA, and atherosclerotic lesions thicker than 4.0 mm at the aortic arch among the three groups. We reviewed medical records for other sources of emboli, such as arterial dissection, ulcerative plaque at the carotid artery, and cardiac and cerebral catheter manipulation.

In the 136 patients having PFO with or without AF, we investigated underlying heart diseases by electrocardiography, TEE and transthoracic echocardiography. Thrombus in lower leg veins was investigated by ultrasonography in 86 patients, by RI scintigraphy in 46 patients, and by either procedure in 93 patients. Using the diagnostic criteria given in Table 1, we ascertained the number of patients who met the definite or probable criteria for paradoxical embolism.

Continuous data were expressed as mean ± SD. We used the Chi squared test for analysis of discrete variables and analysis of variance with the multiple comparison test with Scheffe's test for analysis of continuous variables.

Results

Patients in the PFO-S, PFO-AF, and AF groups were 63.5±11.7 years old, 68.0±11.7 years old, and 70.7±7.8 years old, respectively (ANOVA, $p<0.0001$). Patients in the PFO-S were significantly younger than those in the AF group (multi comparison test with Scheffe, $p<0.0001$, Table 2). Hypercholesterolemia was noted in 54.1%, 20.0%, and 38.8% of the PFO-S, PFO-AF, and AF groups, respectively ($p<0.0001$) and was significantly more frequent in the PFO-S group than the PFO-AF and AF groups ($p<0.0001$).

Infarction occurred in the anterior circulation in 68.5%, 84.0%, and 79.1%, in the posterior circulation in 31.5%, 8.0%, and 11.9%, and in both in 0%, 8.0% and 9.0% of the PFO-S, PFO-AF and AF groups, respectively (Table 2). Patients in the PFO-S group had a significantly higher incidence of lesions restricted to the posterior circulation than did the PFO-AF and AF groups ($p=0.0004$).

Table 1. Diagnostic Criteria for Paradoxical Brain Embolism

1) Brain infarction demonstrated by CT or MRI.
2) Patent foramen ovale diagnosed by TEE.
3) Intravenous thrombus demonstrated by ultrasonography or RI venography.
4) Neuroradiological features of brain embolism,
   such as cortical infarction demonstrated by CT or MRI and angiographic findings of intraluminal filling defect (embolus shadow) or reopening of previously occluded arteries.
5) Absence of other sources of embolism, such as
   heart disease (atrial fibrillation, prosthetic valves, rheumatic heart disease, dilated cardiomyopathy, sick sinus syndrome, acute myocardial infarction, ventricular aneurysm), atherosclerotic plaque at the aortic arch thicker than 4.0 mm, and arterial stenotic lesion (≥50%) proximal to the lesion.

Diagnosis of paradoxical brain embolism.

Definite	1)+2)+3)+4)+5)
Probable	1)+2)+3)+4)
	1)+2)+3)+5)
	1)+2)+4)+5)

Table 2. Demographics

Number	PFO-S group 111	PFO-AF group 25	AF group 67	p
Age (years)	63.5±11.7	68.0±11.7	70.7±7.8*	<0.001
Gender, male	84 (75.7)	22 (88.0)	47 (70.1)	0.21
Hypertension	71 (64.0)	11 (44.0)	45 (67.2)	0.11
Diabetes Mellitus	42 (37.8)	6 (24.0)	24 (35.8)	0.52
Hypercholesterolemia	60 (54.1)	5 (20.0)	14 (20.9)	<0.0001 (<0.0001)+
Territory of the infarction				
Anterior circulation	76 (68.5)	21 (84.0)	53 (79.1)	0.24
Posterior circulation	35 (31.5)	2 (8.0)	8 (11.9)	0.0018** (0.0004)+
Both	0 (0)	2 (8.0)	6 (9.0)	
Stenotic lesion***	31 (22.8)	2 (8.0)	5 (7.5)	0.0011 (0.0002)+
Aortic arch atheroma	10 (9.0)	10 (40.0)	20 (30.0)	<0.0001 (<0.0001)+
Number of patients with infarction located in the anterior circulation.				
	76	23	55	
Infarction >3.0 cm diameter	21 (27.6)	13 (56.5)	34 (61.8)	0.0002 (0.0008)+
Cortical infarction	35 (46.0)	22 (95.7)	46 (83.6)	<0.0001 (<0.0001)+
Number of patients with cerebral angiography.				
	28	13	19	
Embolic shadow	2 (8.8)	2 (15.4)	2 (10.5)	0.71
Intracranial artery occlusion	14 (50.0)	11 (84.6)	16 (84.2)	0.017 (0.0046)+
Follow-up MRA	7	9	7	
Reopening on MRA	0	8 (88.9)	7 (100)	<0.0001 (<0.0001)+

(%), *multi comparison test with Scheffe p<0.0001 vs. PFO-S group, **vs. anterior circulation, ***at the artery proximal to the infarction. +PFO-S group vs. PFO-AF and AF groups.

In patients with infarction in the anterior circulation, lesions larger than 3.0 cm in diameter were seen in 27.6%, 56.5%, and 61.8% (p=0.0002), and cortical lesions were observed in 46.0%, 95.7%, and 83.6% (p<0.0001) of the PFO-S, PFO-AF and AF groups, respectively. Large infarcts and cortical lesions were significantly less frequent in the PFO-S group than in the PFO-AF and AF groups (p=0.0008, p<0.0001, respectively, Table 2).

We performed carotid ultrasonography in all patients, MR angiography (MRA) in 77 (69.4%) of the PFO-S group, in 20 (80.0%) of the PFO-AF group, and in 50 (74.6%) of the AF group. Cerebral angiography was carried out in 28 (25.2%), 13 (52.0%), and 19 (28.4%) of the PFO-S, PFO-AF, and AF groups, respectively. The incidence of arterial stenotic lesion proximal to the infarction was 22.8%, 8.0%, and 7.5% of the PFO-S, PFO-AF, and AF groups, respectively (p=0.0011). The stenotic lesions were significantly more commonly complicated in the PFO-S group than in the PFO-S and AF groups (p=0.0002, Table 2).

The incidence of intracranial arterial occlusion demonstrated by cerebral angiography was 50.0%, 84.6%, and 84.2% (p=0.017), and reopening of a previously occluded artery detected by follow-up MRA was demonstrated in 0%, 88.9%, and 100% (p<0.0001) of the PFO-S, PFO-AF and AF groups, respectively. The incidence of intracranial arterial occlusion and reopening phenomenon was significantly less frequent in the PFO-S group than in the PFO-AF and AF groups (p<0.0043 and p<0.0001, respectively, Table 2). The incidence of embolic shadow was low in all three groups (Table 2). All patients with findings of intraluminal filling defect or reopening phenomenon had cortical infarction.

TEE revealed complicated atheroma at the aortic arch in 9.0%, 40.0%, and 30.0% of the PFO-S, PFO-AF, and AF groups, respectively (p<0.0001). In the 136 patients with PFO, an underlying heart disease was demonstrated in 26 patients (19.1%); non-valvular atrial fibrillation in 25 and sick sinus syndrome in one. Other sources of emboli in the PFO group were ulcerative carotid plaque (n=1), arterial dissection (n=2), and cardiac catheter manipulation (n=1).

In total, sources of emboli including a cardiac source of emboli (n=26), cerebral artery stenosis ≥50% (n=33), complicated aortic atheroma (n=20), and other sources mentioned above (n=4) except for PFO and deep vein thrombosis were demonstrated in 72 (52.9%) of the 136 patients with PFO (eight had both AF and aortic atheroma, one had both AF and stenotic lesion, and one had AF, stenotic lesion and aortic atheroma). Deep vein thrombosis was found in 25 of the 93 patients (26.9%) who were examined by ultrasonic examination or RI scintigraphy. Of these 93 patients, the definite and probable criteria for paradoxical brain embolism were fulfilled in only 3 (3.2%) and 33 cases (35.5%), respectively.

Discussion

Several studies have revealed that paradoxical embolism through PFO is an important stroke mechanism (2–4). However, in the present study, we found that 3.2% and 35.5% of stroke patients with PFO fitted the criteria for definite and probable paradoxical brain embolism, respectively. We also found that a considerable number of stroke patients with PFO had other sources of emboli (52.9%) and risk factors of atherosclerosis. Neuroradiological features of embolic stroke such as large or cortical infarction, or reopening of a previously occluded artery were less common in the PFO-S group than in the PFO-AF and AF groups. On the other hand, the clinical and neuroradiological features in the PFO-AF group were similar to those in the AF group. These distinguishing characteristics of the PFO-S and PFO-AF groups suggest that a considerable number of patients developed stroke not only by paradoxical embolism through PFO but also by other embolic mechanisms from a cardiac source, proximal arterial stenosis, atherosclerotic lesions in the aortic arch, or thrombotic or hemodynamic mechanisms in the large or small arteries. Therefore, the risk of stroke and other sources of emboli in stroke patients with PFO must be investigated to determine if they meet the criteria for paradoxical embolism, which requires anticoagulant therapy against recurrent attacks. The present study was retrospective, and thus prospective studies examining consecutive stroke patients are required to obtain an accurate prevalence rate for paradoxical embolism in stroke.

PFO is an important mechanism by which stroke develops in the young (2, 3), whereas in the elderly, non-valvular atrial fibrillation (NVAF) is the most frequent embolic source of brain infarction (12). Recent population-based surveys have revealed that 10% of people over 80 have AF (13). Thus, the differences in several features of stroke patients among the PFO-S, PFO-AF, and AF groups may be reflected by a difference in age.

Infarction in the posterior circulation was common in the PFO-S group. Small emboli passing through the PFO may enter the vertebral arteries more easily than the common carotid arteries. Otsubo et al reported that aortogenic infarction tends to occur at the posterior circulation (14). Therefore, emboli from atherosclerotic lesions in the aortic arch may play an important role in developing stroke in the PFO-S group, although aortic atherosclerotic lesions were also reported to play an important role in the development of stroke in patients with NVAF (15).

Exploration for deep vein thrombus is essential for proper diagnosis of paradoxical embolism. The detection rate of thrombus was 26.9% among the cases investigated in the present study. Recently, echo examination was applied to small veins for detecting thrombi. The more widely the echo examination is applied, the higher the detection rate of venous thrombi in stroke patients with PFO. If thrombi are detected, anticoagulant therapy should be applied and if not, antiplatelet treatment may achieve prevention to the same extent as the anticoagulant therapy (16).

In conclusion, the clinical features of patients having PFO with sinus rhythm appear to differ from those of patients

with AF. Other causes of stroke should be considered in stroke patients with PFO because not only paradoxical brain embolism through the PFO but also other causes of stroke may contribute to development of the stroke.

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Clinical Characteristics of Stroke Patients with Antiphospholipid Antibodies

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Key Words
Antiphospholipid syndrome · Cerebral infarction

Abstract
Background: Antiphospholipid syndrome is important as a cause of ischemic stroke, although clinical characteristics of the syndrome are not well documented. ***Methods:*** We analyzed differences in clinical characteristics between 40 antiphospholipid-antibody (aPL)-positive and 40 aPL-negative stroke patients. ***Results:*** Stroke patients with aPL were significantly younger and were more likely to be women in comparison with stroke patients without aPL. Valvular heart disease, neurological complications and hematological disorders were more frequent in the aPL-positive group. The mean value of thrombin-antithrombin III complex was significantly lower in the aPL-positive group. Cerebral infarctions in the carotid system were less and large-artery lesions more frequent in the aPL-positive patients. ***Conclusions:*** Stroke patients with aPL have clinical characteristics distinct from stroke patients without aPL.

Introduction

Antiphospholipid syndrome (APS) is an acquired coagulopathy characterized by antiphospholipid antibodies (aPL), vascular thrombosis and recurrent pregnancy loss. APS is also important as a cause of ischemic stroke. For example, it is known that aPL are a causative factor for stroke in a large number of young stroke patients. Although a great variety of clinical features have been described in patients with APS, there are few reports describing the clinical differences between aPL-positive and aPL-negative stroke patients [1]. The aim of the present study is to analyze the clinical characteristics of aPL-positive stroke patients and compare them with those of aPL-negative stroke patients. We compared gender, age, stroke risk factors, associated diseases, hemostatic markers and radiological findings between aPL-positive and aPL-negative patients with acute ischemic stroke.

Patients and Methods

Between 1996 and 2001, we enrolled 40 consecutive aPL-positive patients with acute ischemic stroke admitted to the Department of Neurology, Tokyo Women's Medical University. Patients fulfilled the Sapporo criteria for diagnosis of APS [2]. Patients with aPL were required to show anticardiolipin IgG antibodies (aCL

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IgG), anticardiolipin IgM antibodies (aCL IgM), anticardiolipin β_2-glycoprotein I antibodies (aCL β_2-GPI) and/or lupus anticoagulant on at least 2 examinations 6 weeks apart. The presence of aCL IgG and IgM isotypes was measured by a β_2-GPI-dependent enzyme-linked immunosorbent assay (ELISA; Mesacup Cardiolipin Test, Medical and Biological Laboratories Co., Nagoya, Japan). The aCL β_2-GPI titer was also measured by ELISA (aCL β_2-GPI kit, Yamasa, Choshi, Japan). Lupus anticoagulant activity was detected by coagulation assays utilizing activated partial thromboplastin time or kaolin clotting time, adhering to the Sapporo criteria. Forty consecutive aPL-negative stroke patients who were admitted during the same period were enrolled as controls.

We analyzed differences in gender, age, risk factors for stroke, hemostatic markers and radiological findings between the aPL-positive and aPL-negative groups. With regard to risk factors for stroke, prevalences of hypertension, hyperlipidemia and diabetes mellitus were compared between the two groups. We also compared neurological manifestations and associations of coagulopathy, venous thrombosis and systemic lupus erythematosus (SLE) and other collagen vascular diseases between the two groups.

Hemostatic markers examined in plasma were β-thromboglobulin (β-TG), platelet factor 4 (PF4), thrombin-antithrombin III complex (TAT) and D-dimer. β-TG was measured using an Asserachrom β-TG kit (Diagnostica Stago, Asnières, France). PF4 was also measured using an Asserachrom PF4 kit (Diagnostica Stago). TAT was measured using a TAT SRL kit (SRL, Tokyo, Japan), and D-dimer was measured using an Lpia-ace D-dimer kit (Mitsubishi Kagaku Iatron, Tokyo, Japan). These hemostatic markers were quantitated using ELISA.

Hypertension was defined as systolic blood pressure above 140 mm Hg or diastolic blood pressure above 90 mm Hg on admission, or a history of high blood pressure requiring medical treatment. The diagnosis of diabetes mellitus was made if a patient was treated with oral glucose depressants or insulin, or if their glycosylated hemoglobin levels were 6.5% or higher, based on the criteria of the Japan Diabetes Mellitus Society [3]. Hyperlipidemia was defined as an elevated fasting serum total cholesterol level more than 220 mg/dl or a history of hyperlipidemia requiring statin treatment.

In order to evaluate cardiac diseases such as valvular heart disease, arrhythmia and patent foramen ovale, we performed electrocardiography, Holter cardiac monitoring, transthoracic echocardiography and transesophageal echocardiography. Brain MRI was performed in all patients to determine the site and number of cerebral infarctions. Multiple cerebral infarctions were diagnosed when more than 3 infarcts were detected. Patients with more than 50% stenosis in the carotid and/or intracranial major arteries on either MR angiography or carotid duplex ultrasonography were defined as having large-artery lesions.

Differences between the two groups were analyzed using χ^2 tests for nominal variables with 5 or more possible values and Fisher's exact test for nominal variables with fewer than 5 possible values. Student's independent t test was used for comparing continuous variables. We also used the Mann-Whitney test for comparing hemostatic markers because of their skewed distributions. Two-sided p values were calculated, and $p < 0.05$ was chosen for statistical significance. Statistical analysis was performed using the SPSS software package (version 7.5, SPSS Inc., Chicago, Ill., USA).

Table 1. Gender, age and aPL in aPL-positive and -negative groups

	aPL		p value
	positive (n = 40)	negative (n = 40)	
Gender			0.007[1]
Men	12	25	
Women	28	15	
Mean age, years	46.9	66.2	<0.0001[2]
aCL IgG	13	0	
aCL IgM	16	0	
Lupus anticoagulant	14	0	
aCL β_2-GPI	12	0	

[1] χ^2 test.
[2] Student unpaired t test.

Results

Age, Gender and aPL

Age, gender and aPL of the two groups are shown in table 1. Distributions according to gender and age differed significantly between the groups. Stroke patients with aPL were significantly younger (46.9 vs. 66.2 years, $p < 0.0001$) and significantly more likely to be women (70 vs. 37.5%, $p = 0.007$) in comparison with stroke patients in the aPL-negative group.

Stroke Risk Factors

Differences in the prevalence of stroke risk factors between the aPL-positive and aPL-negative patients with acute ischemic stroke are shown in table 2. Diabetes mellitus was less frequent in the aPL-positive group (18%) than in the aPL-negative group (43%; $p = 0.028$). There were no significant differences in the prevalence of hypertension, hyperlipidemia or cardiac disease between the two groups. However, when the prevalence of valvular disease was analyzed separately from other cardiac diseases, its proportion differed significantly between the groups. Thus, in the aPL-positive group 58% of the patients had valvular disease, whereas only 13% of the patients in the aPL-negative group had valvular heart disease ($p = 0.0001$). The mean number of stroke risk factors per patient (other than aPL) differed significantly between the groups. The mean numbers of risk factors in the aPL-positive and aPL-negative groups were 1.0 and 1.65, respectively ($p = 0.007$). It is noteworthy that 20 patients (50%) in the aPL-positive group had

Table 2. Differences in prevalence of risk factors between aPL-positive and -negative patients with cerebral infarction

Risk factor	aPL		p value[1]
	positive (n = 40)	negative (n = 40)	
Hypertension	12 (30)	18 (45)	NS
Hyperlipidemia	6 (15)	12 (30)	NS
Diabetes mellitus	7 (18)	17 (43)	0.028
Heart disease	18/23 (90)	14/40 (35)	0.002
Arrhythmia	10 (43)	12 (30)	NS
Valvular disease	14 (61)	4 (13)	0.0001
Mean number of risk factors	1.00	1.65	0.007[2]

Figures in parentheses are percentages. NS = Not significant.
[1] χ^2 test.
[2] Student's unpaired t test.

Table 3. Differences in clinical features between aPL-positive and -negative patients with cerebral infarction

Clinical feature	aPL		p value[1]
	positive (n = 40)	negative (n = 40)	
Neurological complication[2]	18 (45)	0	0.0001
Hematological disorder[3]	12 (30)	3 (8)	0.022
Venous thrombosis	6 (15)	0	0.034
All collagen vascular diseases	9 (23)	3 (8)	NS
SLE	6 (15)	0	0.034

Figures in parentheses are percentages. NS = Not significant.
[1] χ^2 test.
[2] Includes epilepsy, myelitis and migraine.
[3] Includes protein S and C deficiencies and thrombocytopenia.

no risk factors other than aPL. In contrast, only 3 (7.5%) patients in the aPL-negative group had no stroke risk factors.

Neurological Manifestations

Differences in clinical features between the groups are shown in table 3. Eighteen (45%) patients in the aPL-positive group showed neurological complications other than stroke, such as epilepsy, myelitis or migraine, whereas no patient in the aPL-negative group had any neurological complications ($p < 0.0001$). Coagulopathies, including protein C and/or protein S deficiency and thrombocytopenia, were observed in 30 and 8%, respectively, in the aPL-positive and aPL-negative patients with acute ischemic stroke ($p = 0.022$). Six patients in the aPL-positive group, as opposed to none in the aPL-negative group, suffered from deep vein thrombosis ($p = 0.034$). Considering all collagen vascular diseases together, no significant difference was observed between the groups. However, SLE was present in 6 aPL-positive patients but in none of the aPL-negative patients ($p = 0.034$).

Hemostatic Markers

There was no significant difference in the mean value of β-TG, PF4 or D-dimer between the aPL-positive and aPL-negative groups (table 4). Only the mean value of TAT was significantly lower in the aPL-positive group than in the aPL-negative group (26.5 vs. 38.2 ng/ml, $p = 0.012$).

Table 4. Hemostatic markers (ng/ml) in aPL-positive and -negative patients with cerebral infarction

Markers	aPL		p value[1]
	positive (n = 40)	negative (n = 40)	
β-TG	138.6 ± 107	142.6 ± 125	NS
PF4	63.4 ± 77	61 ± 70	NS
TAT	5.8 ± 15	6.6 ± 6	0.012
D-dimer	53.0 ± 57	65.5 ± 99	NS

Data are shown as means ± 1 standard deviation. NS = Not significant.
[1] Mann-Whitney U test.

Radiological Findings

As shown in table 5, cerebral infarctions in the carotid system were less frequent in the aPL-negative patients as compared to the aPL-positive patients (21 vs. 33, i.e. 52.5 vs. 82.5%, $p = 0.009$). No significant differences were observed between the two groups in the prevalence of cortical and multiple infarcts. Large-artery lesions defined as more than 50% stenosis in the carotid and/or intracranial major arteries on either MR angiography or carotid ultrasonography were detected in 14 (35%) and 34 (85%) of the aPL-positive and aPL-negative patients, respectively ($p = 0.038$).

Table 5. Radiological findings in aPL-positive and -negative patients with cerebral infarction

Radiological finding	aPL		p value[1]
	positive (n = 40)	negative (n = 40)	
Infarction in carotid system	21 (53)	33 (83)	0.009
Infarction in vertebrobasilar system	18 (45)	15 (38)	NS
Cortical infarction	13 (33)	11 (28)	NS
Multiple infarcts	28 (70)	20 (50)	NS
Large-artery lesion[2]	14 (35)	34 (85)	0.038

Figures in parentheses are percentages. NS = Not significant.
[1] χ^2 test.
[2] More than 50% stenosis in the carotid and/or intracranial major arteries on either MR angiography or ultrasonography.

Discussion

aPL are frequently observed in patients with SLE and are associated with an increased risk of thrombosis. aPL are also detected in patients with other collagen vascular diseases, infectious diseases and malignancies. In addition, it has been reported that aPL can be positive even among 1–5% of the normal population [4]. Previous studies have reported that aPL are present in 9.7–29% of stroke patients without any collagen disease [5, 6]. The Antiphospholipid Antibodies in Stroke Study (APASS) Group [7] has shown that the odds ratio for stroke in patients with positive aPL is significantly higher than 1 (2.31) after adjustment for other stroke risk factors, indicating that aPL are an independent risk factor for stroke [8].

It is recognized that APS is a female-predominant disorder that tends to occur in relatively younger patients, and 13–30% of patients with APS develop stroke later [1, 9]. Levine et al. [10] showed that the mean age of patients with aPL-positive stroke was 43 years, and the male:female ratio was 1:2 in 48 patients. The mean age of aPL-positive stroke patients in the present study was 47 years, and 70% of them were women.

Common risk factors for stroke have been reported to be less frequent in aPL-positive than aPL-negative stroke patients [11]. In our study, diabetes mellitus was significantly less common in the aPL-positive than aPL-negative stroke patients. The mean number of risk factors was also significantly lower in the aPL-positive group. Furthermore, 50% of the aPL-positive patients in our study had no other stroke risk factors. Levine et al. [10] speculated that the immunological consequences of aPL might account for the development of stroke in this group of patients. Our results suggest that immune-mediated thrombogenesis arising from aPL increases stroke risk more than common risk factors for atherosclerosis in these patients.

Several mechanisms have been proposed for the development of thrombosis in APS. These mechanisms include fibrinolysis, antithrombin III, prostacyclin generation, platelet aggregability, functional alterations of protein C and complement activation [12, 13]. Furthermore, disruption of the annexin V antithrombotic shield is one of the interesting hypotheses proposed by Rand and Wu [14]. This hypothesis offers the first simultaneous explanation for prolonged coagulation time and thrombophilic tendency. Thus, it has been proposed that some of the above-mentioned mechanisms of thrombogenesis play distinct roles in the pathogenesis of stroke in aPL-positive patients.

The prevalence of cardiac disease, another risk factor for stroke, was significantly different between aPL-positive and aPL-negative stroke patients in the present study. In particular, the prevalence of valvular disease was significantly higher in the aPL-positive group. Vianna et al. [15] showed that 63% of patients with aPL-positive stroke had valvular disease. Khamashta and Hughes [16] showed that 23% of SLE patients had some valvular lesions, and these valvular diseases were strongly correlated with the presence of aCL. These reports suggest that aCL can be a risk factor for valvular heart disease in patients with SLE and APS. The pathogenesis of valvular disease has been hypothesized to involve fibrin thrombi on the valve and its organization leading to fibrosis and dysfunction, as in cases of Libman-Sacks endocarditis [17]. aPL, even without SLE, may play a role in valvular damage by thrombus formation on the endocardium [18].

Atsumi and Koike [19] reported that more than 90% of arterial events were cerebrovascular in APS patients [20]. Ischemic heart disease is rare, but valvular disease is frequent, which correlates with cerebrovascular thromboembolic complications in APS patients. By using transcranial Doppler ultrasound, Rademacher et al. [21] detected microembolic signals in 39% of patients with APS, whereas none were detected in those without APS among SLE patients. They concluded that microembolic signals may be a useful indicator of active APS and that subsequent stroke can be predicted by microembolic signals. Our results, together with these reports, imply that stroke in APS patients is caused not only by in situ im-

mune-mediated mechanisms linked to aPL, but also by cardioembolic mechanisms due to valvular disease. These conditions frequently coexist in aPL-positive patients. Thus, it should be emphasized that careful cardiac examination to detect sources of emboli is crucial for therapeutic decision making.

In the present study, stroke patients in the aPL-positive group had more frequent neurological complications such as epilepsy, myelitis or migraine than those in the aPL-negative group [22, 23]. The association of migraine and APS is controversial, with widely varying results from different series. In this study, 8 stroke patients in the aPL-positive group had migraine, but none in the aPL-negative group. Our results support a relationship between migraine and APS, although the sample size is small and statistical power is weak. Furthermore, one fourth of the aPL-positive patients in this study suffered from deep vein thrombosis, which is very common in patients with APS [24]. Up to half of these patients had pulmonary emboli [25]. Therefore, it is important for physicians to consider APS as a component of the differential diagnosis when patients with stroke present with one or more of the above-mentioned neurological symptoms.

Hematological abnormalities such as protein C and S deficiencies as well as thrombocytopenia were more frequent in our aPL-positive than aPL-negative patients. Some aPL-positive patients have antibody activity against protein C and protein S. Therefore, it is possible that thrombosis due to decreased protein C and protein S antigens, together with immunological mechanisms, might contribute to the development of stroke in patients with APS [26].

As for coagulation markers such as β-TG, PF4 and D-dimer, no significant differences could be seen between the aPL-positive and aPL-negative groups. Because platelet activation is frequently observed in the acute phase of stroke irrespective of aPL, increases in β-TG and PF4 are common in both aPL-positive and aPL-negative patients [27]. Such responses may explain why we found no significant differences in β-TG or PF4 between the groups. In contrast, the mean values of TAT differed between the two groups. TAT in the aPL-positive group was lower than that in the aPL-negative group. Yamazaki et al. [28] reported that there was no elevation of TAT in patients with APS. Ieko [29] speculated that the existence of abundant free thrombin due to inhibition of TAT formation could contribute to thrombosis in APS. Naitoh et al. [30] suggested that inhibition of the formation of TAT is caused mainly by aCL IgG. Shibata et al. [31] reported that aCL IgG antiheparin antibodies inhibit the heparin-accelerated formation of TAT. They concluded that antiheparan sulfate/heparin antibodies may be a cause of vascular thrombosis in APS. These studies support the results in our study. Our results revealed that many patients with aPL-positive stroke do not show any increase in TAT.

Results of previous radiological studies have suggested that stroke patients with APS often have multiple infarcts present in the white matter or basal ganglia, and in cortical or subcortical areas [32–34]. In the present study, neither the number of lesions nor the percentage of cortical infarcts differed significantly between the two groups. One possible explanation is that all patients in the control (aPL-negative) group required hospitalization due to relatively severe infarction compared with patients treated in an outpatient clinic. Specifically, patients in our control group tended to have multiple and/or large cortical infarcts, which might have obscured differences between the two groups. As for the location of infarcts, infarcts in the carotid system were less frequent in the aPL-positive group. This also means that infarcts in the vertebrobasilar system were more common in patients with aPL than in patients without aPL. Five patients in the aPL-positive group showed basilar syndrome that is probably attributable to a cardioembolic mechanism [19, 21]. There are several reports concerning angiographic findings in patients with APS [35, 36]. The APASS Group reported that 36.7% of APS patients had normal angiographic studies and 49.0% showed intracranial arterial lesions on cerebral angiography [9]. In the present study, we evaluated arterial lesions by using MR angiography and carotid duplex ultrasonography. Large arterial lesions were less frequent in the aPL-positive group. It has been suggested that thrombosis in APS is caused partly by oxidized low-density lipoprotein, which accelerates atherosclerosis [37]. However, if the stroke subtype in APS was cardioembolic, it may develop without progression of atherosclerosis in major arteries. In addition, according to the definition of APS, APS-induced stroke has to be stroke without underlying angiitis [2]. Therefore, our results indicate that stroke occurring without obvious arterial lesions is one of the relevant characteristics of APS.

In conclusion, stroke with aPL is associated with several patient characteristics: (1) a higher incidence in females than males and onset at a relatively young age, (2) frequent association with valvular heart disease, (3) less frequent prevalence of the common risk factors for stroke such as hypertension, hyperlipidemia and especially diabetes mellitus, (4) frequent neurological or hematological

complications such as epilepsy, myelitis, migraine, coagulopathy, deep vein thrombosis and thrombocytopenia, (5) less prominent elevation of TAT, (6) preferential involvement of the vertebrobasilar system and (7) less frequent large-artery lesions. The small size and potential bias that could exist in the aPL-positive stroke patients limit this study. Results of the present study should be further confirmed in a future study with a large patient population.

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Associations of Serum IL-18 Levels With Carotid Intima-Media Thickness

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Objective—Elevated circulating levels of IL-18 can predict future coronary heart disease. Although IL-18 is thought to play a crucial role in atherosclerosis, whether circulating IL-18 levels are associated with the severity of atherosclerosis remains to be determined. With the use of B-mode ultrasound, this study examines the relationships of serum IL-18 levels with carotid intima-media thickness (IMT) as a reflector for systemic atherosclerosis.

Methods and Results—The study comprised 366 patients without histories of cardiovascular accidents. Severity of carotid atherosclerosis was evaluated by the mean max IMT, ie, mean of the maximal wall thickness at 12 carotid segments. Serum IL-18, IL-6, and high-sensitive C-reactive protein (hs-CRP) levels were determined in all patients. Log-transformed IL-18 concentrations were positively correlated with IMT (r=0.36, P<0.001), and the association remained significant (β=0.20, P<0.001) when controlling for traditional atherosclerotic risk factors, IL-6 and hs-CRP levels. Also, IMT was greater in the highest and the middle tertile of IL-18 levels than in the lowest tertile.

Conclusion—Higher serum IL-18 levels appear to be associated with greater carotid IMT, suggesting the link between IL-18 and atherosclerosis. (***Arterioscler Thromb Vasc Biol.* 2005;25:1458-1462.**)

Key Words: atherosclerosis ■ carotid arteries ■ cytokines ■ inflammation ■ ultrasonic diagnosis

With the recognition that inflammation plays a significant role in atherosclerosis and its complications, studies have shown that circulating inflammatory markers are predictive of cardiovascular diseases (CVD).[1] Particularly, elevated circulating levels of C-reactive protein (CRP) and IL-6 (IL-6) have been associated with the risk for CVD.[2–4] Additionally, circulating levels of IL-18 are found to be a strong predictor of CVD deaths in patients with coronary artery disease,[5] as well as an independent predictor for coronary events in healthy men.[6]

IL-18 was originally identified as an interferon-γ–inducing factor,[7] which may play a central role in the inflammatory cascades.[8] To date, experimental studies have shown that expression of IL-18 is related with atherosclerotic plaque progression and its instability.[9,10] However, clinical studies relating IL-18 to atherosclerotic severity are limited.[11] Moreover, we are unaware of studies that examined the relationships with IL-6 and high-sensitive CRP (hs-CRP) taken into account.

Carotid intima-media thickness (IMT), as assessed by B-mode ultrasound, is a commonly used clinical marker that reflects systemic burden of atherosclerosis.[12] Moreover, increased IMT has been a predictor of future coronary events and stroke.[13–15] On the basis of such findings, to clarify the relationships between such inflammatory markers and IMT would be of value to extend the current knowledge regarding the link between inflammation and atherosclerotic diseases.

With the use of B-mode ultrasound, this study examines the relationships of serum IL-18 levels with carotid IMT as a reflector for systemic atherosclerosis.

Methods

Subjects

The subjects for this investigation were enrolled from patients of the Department of Internal Medicine and Therapeutics at Osaka University Hospital, who had undergone carotid ultrasound examination between October 2000 and November 2004.

In the current study, patients with the histories of ischemic heart disease, stroke or arteriosclerosis obliterans were excluded, because circulating IL-18 levels can be substantially modified in such patients.[16–18] Also, patients with acute inflammatory diseases, collagen diseases, or malignant neoplasm were excluded, because levels of inflammation can be greatly enhanced by such diseases. Additionally, patients with occluded carotid arteries and those with the history of carotid endarterectomy were excluded, because IMT could not be correctly determined in such patients.

Consequently, this study comprised 366 patients (mean±SD, age 64.8±9.1 years) equally including men and women. Patients' characteristics are shown in Table 1, demonstrating higher prevalence of atherosclerotic risk factors in the study sample. Institutional ethical committee approved this study, and written informed consent was obtained from all patients. Also, the investigation conforms to the principles outlined in the Declaration of Helsinki.

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TABLE 1. Patient Characteristics (n=366)

Age, y	64.8±9.1
Men, n (%)	180 (49)
Body mass index, kg/m^2	23.2±2.8
Hypertension, n (%)	262 (72)
Medical treatment, n (%)	195 (53)
ACEI or ARB use, n (%)	80 (22)
Systolic blood pressure, mm Hg	137±18
Diastolic blood pressure, mm Hg	80±12
Diabetes mellitus, n (%)	64 (18)
Medical treatment, n (%)	26 (7)
Fasting blood glucose, mmol/L	5.7±1.3
Dyslipidemia, n (%)	255 (70)
Medical treatment, n (%)	98 (27)
Statin use, n (%)	80 (22)
Total cholesterol, mmol/L	5.6±0.9
Triglyceride, mmol/L	1.5±0.8
HDL-cholesterol, mmol/L	1.5±0.4
Smokers, n (%)	169 (46)
Aspirin use, n (%)	45 (12)
Inflammatory markers	
IL-18, pg/mL (median)	194±81 (177)
IL-6, pg/mL (median)	1.96±2.38 (1.41)
hs-CRP, mg/dL (median)	0.13±0.23 (0.06)
Mean max-IMT, mm	0.99±0.27

Error terms are SD. ACEI indicates angiotensin-converting enzyme inhibitors; ARB, angiotensin II type1 receptor blocker.

Carotid Ultrasonography

All ultrasound examinations were performed with the use of Phillips SONOS 5500 equipped with a 3- to 11-MHz linear-array transducer. Three different longitudinal (anterior oblique, lateral, and posterior oblique) and transverse images of the bilateral carotid arteries were obtained, and IMT was measured as the distance between the luminal–intimal interface and the medial–adventitial interface. It was measured with the use of an electronic caliper on the frozen frame of a suitable longitudinal B-mode image in which putative maximal IMT was visualized. Thereby, severity of carotid atherosclerosis was evaluated by the mean max-IMT, which is the mean of maximal wall thickness at 12 carotid segments (near and far wall of the left and right common carotid artery, carotid bifurcation, and internal carotid artery).

All examinations were performed by one sonographer (H.Y.) who was blinded from the patients' clinical details. Before this study, reproducibility of the mean max-IMT was examined for randomly selected 70 patients without carotid occlusion, in which IMT measurements were performed twice by the same examiner. Intraclass correlation coefficient for the mean max-IMT measurements was 0.96, with a similar average between the two measurements.

Measurement of Serum Inflammatory Markers

After carotid ultrasound examinations, blood was drawn with minimally traumatic venipuncture for the measurement of serum inflammatory markers. Thereafter, the blood was centrifuged at 3000 rpm at 4°C for 15 minutes, and aliquots were stored at −70°C. Serum concentration of IL-18 was measured by single determination with enzyme-linked immunosorbent assay method (MBL Co, Ltd. Nagoya, Japan). In this assay system, mean interclass coefficient of variation (CV) of IL-18 measurements was 5.9%. Also, in 52 randomly selected patients, within-person correlation coefficient by 1-year interval was 0.84 ($P<0.001$).

Additionally, level of IL-6 was measured by enzyme-linked immunosorbent assay method (R & D system, Minneapolis, Minn), and hs-CRP was measured by latex turbidimetric immunoassay (Shionogi Biomedical Laboratory Inc, Osaka, Japan).

Evaluation of Atherosclerotic Risk Factors

Levels of fasting blood glucose, serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides (TG) were determined from the blood sample taken for inflammatory marker evaluations. Information on the patients' medical histories and medication usages was obtained from the clinical records. Hypertension was defined by casual blood pressure ≥140/90 mm Hg or the current use of antihypertensive agents. Diabetes mellitus was defined by fasting blood glucose ≥7.0 mmol/L or by the use of glucose-lowering agents. Dyslipidemia was defined by fasting serum total cholesterol >5.7 mmol/L, TG >1.7 mmol/L, HDL cholesterol <1.04 mmol/L, or by the use of cholesterol-lowering agents. Smoking status was categorically evaluated based on self-reports, with a smoker defined by the history of smoking ≥10 cigarettes per day >1 year.

Statistical Analyses

All analyses were performed with SPSS 11.5J (SPSS Japan Inc). Because distributions of inflammatory markers levels appeared to be left-skewed, they were normalized by log-transformation. Thereafter, associations between IL-18 levels and atherosclerotic risk factors were examined by Pearson correlation analysis for continuous variables, and by unpaired t test for categorical variables. Also, relationships between inflammatory marker levels and mean max-IMT were examined by Pearson correlation analysis. Subsequently, multiple linear regression analyses were used to examine associations between IL-18 levels and mean max-IMT: (1) by controlling for age and sex; (2) by additionally controlling for traditional atherosclerotic risk factors (body mass index, hypertension, diabetes, smoking status, total cholesterol, TGs, and HDL cholesterol); and (3) by further controlling for IL-6 and hs-CRP levels. Finally, mean max-IMT was compared across the IL-18 tertiles by the general linear model, followed by Bonferroni post-hoc test. Probability values were 2-tailed and were considered significant when <0.05.

Results

Associations between IL-18 levels and atherosclerotic risk factors are shown in Table 2. Levels of IL-18 were higher in men than in women, in patients with hypertension than in those without, and in patients with smoking history than in those without. Also, IL-18 was positively correlated with age, body mass index, and TGs, and negatively with HDL cholesterol. Additionally, IL-18 levels showed modest correlations with IL-6 and hs-CRP ($r=0.23$ and 0.29; both $P<0.001$). Of note, IL-18 levels were similar between patients on aspirin, statins, angiotensin-converting enzyme inhibitors, or angiotensin II type 1 receptor blocker, and those not using them (data not shown).

To clarify the link between IL-18 and severity of atherosclerosis, associations of serum IL-18 levels with the mean max-IMT were examined. By univariate analysis, log-transformed concentration of IL-18 was positively correlated with IMT ($r=0.36$, $P<0.001$). By multiple regression analyses (Table 3), the association between IL-18 and IMT remained significant when controlling for age and sex (model 1), and additionally controlling for traditional atherosclerotic risk factors (model 2). Moreover, the association was little attenuated when further controlling for IL-6 and hs-CRP (model 3). Of note, although both IL-6 and hs-CRP had