reported that the use of sonography with t-PA therapy improved outcomes in patients with hyperacute ischemic stroke. Therefore, in the near future, TCCS may be useful not only as a diagnostic tool but also as a treatment in patients with MCA disease.

Conclusion

To our knowledge, we are the first group to develop TCCS criteria for diagnosing MCA stem occlusion and MCA branch occlusion. TCCS is a useful tool in the assessment of MCA diseases in patients with acute stroke.

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Link between Linear Hyperintensity Objects in Cerebral White Matter and Hypertensive Intracerebral Hemorrhage

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Key Words
Linear hyperintensity objects · Cerebral white matter · MRI · Intracerebral hemorrhage · Hypertension

Abstract
Background: We retrospectively studied the relationship between linear hyperintensity objects (LHOs) on T_2-weighted magnetic resonance images (MRI) in the cerebral white matter and the occurrence of hypertensive intracerebral hemorrhage (HIH). ***Methods:*** Forty-nine hypertensive patients with a fixed imaging condition MRI were classified into three groups: HIH (n = 17), ischemic stroke due to hypertensive vasculopathy (n = 19), and hypertension only (n = 13). After assessing clinical and radiological background information among these groups and the reliability of LHO measurements, polynomial logistic regression analysis was used to identify the factors relating to HIH. ***Results:*** HIH had a significantly higher LHO number (p = 0.002) and larger diameter (p = 0.007). The LHO number showed an excellent interrater (κ = 0.91, 95% CI = 0.87–0.94, SEM = 6.2%) and intrarater reliability (κ = 0.95, 95% CI = 0.92–0.97, SEM = 4.8%), and was the most significant independent indicator of HIH (OR = 1.29, 95% CI = 1.05–1.60, p = 0.017). The number of microbleeds was an additional indicator (OR = 3.73, 95% CI = 1.10–12.65, p = 0.034). ***Conclusions:*** LHOs are closely linked to HIH. A prospective, longitudinal study is needed to clarify whether LHOs can predict HIH.

Introduction

Hypertensive intracerebral hemorrhage (HIH) is widely regarded as the deadliest of stroke subtypes. Unfortunately, there is no comprehensive method for detection of HIH in most hypertensive patients, except for microbleeds on T_2- or T_2*-weighted MRIs of patients with ischemic stroke or who take antithrombotic medication [1–4]. We recently found that linear hyperintensity objects (LHOs) on T_2-weighted MRIs of cerebral white matter are more prominent depending on the severity of hypertension, and may be based on both hypertensive arteriolar vasculopathy of the white matter medullary artery and its dilated perivascular spaces [5, 6]. Notably, most of the patients with prominent LHOs were noted to have suffered from HIH (fig. 1). Therefore, it is speculated that

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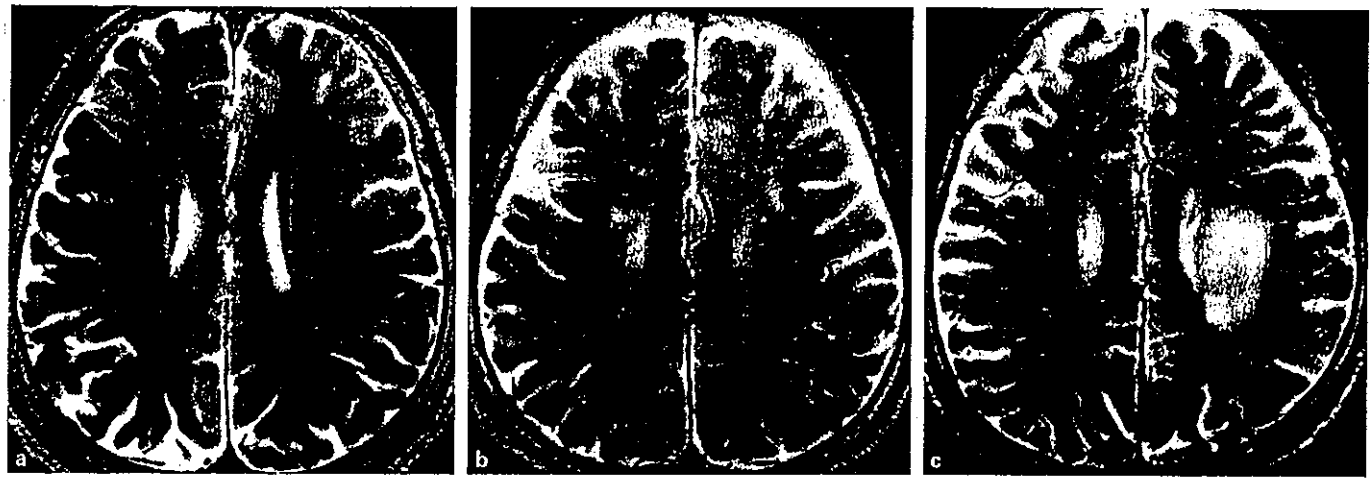

Fig. 1. Typical LHOs in hypertension-only (**a**), ischemic stroke due to hypertensive vasculopathy (**b**), and HIH (**c**). Case A: A 48-year-old patient had hypertension of 12 years' duration and no organic lesions in any area of the brain. Subtle LHOs are seen in the cerebral white matter. Case B: A 53-year-old patient had hypertension of 13 years' duration and multiple small infarcts in the basal ganglia and corona radiata. LHOs are less apparent. Case C: A 37-year-old patient had hypertension of 8 years' duration and left putaminal hemorrhage. Prominent LHOs are seen in the bilateral cerebral white matter.

LHOs and HIH may share a common pathological background as hypertensive microangiopathy, and that LHOs may be a promising indicator of HIH. Since the detection of details such as LHOs strongly depends on the imaging condition [7], we retrospectively and cross-sectionally studied hypertensive patients who underwent magnetic resonance imaging (MRI) with a fixed-imaging condition, focusing on the reliability of our manual evaluation of LHOs, and on the relationship between the severity of LHOs and the presence of HIH, taking the clinical and radiological backgrounds into consideration.

Materials and Methods

Subject Selection and Classification

A brain MRI with a fixed-imaging condition was performed on 274 consecutive patients admitted to the Department of Neurology at the Tokyo Metropolitan Neurological Hospital between May 1996 and April 1998. Patients with various brain disorders routinely underwent a brain MRI in our Department. The clinical diagnosis of the 274 patients was as follows: cerebrovascular disease in 105, infectious disease in 19, spinocerebellar degeneration in 18, motor neuron disease in 18, multisystem atrophy in 16, Parkinson's disease in 12, multiple sclerosis in 12, progressive supranuclear palsy in 7, and other disorders in the remaining 67 patients. From this group of patients, we selected all of the 87 patients with hypertension and grouped them into the following: HIH, ischemic stroke, transient ischemic attack, and hypertension only (fig. 2). Excluding patients whose MRIs were missing or had severe motion artifacts, 17 patients were selected for the HIH, 38 patients for the ischemic stroke, and 19 patients for the hypertension-only groups. Based on the WHO classification of severity of hypertensive organopathy in brain, heart, optic fundi, and kidney [8], we selected 31 patients with ischemic stroke due to hypertensive vasculopathy from the ischemic stroke group. Finally, to match the HIH patients for age, gender, duration of hypertension, and left ventricular hypertrophy, we grouped 19 patients into ischemic stroke due to hypertensive vasculopathy and 13 patients into hypertension only.

The diagnosis of HIH was made using all three clinical presentations including hypertensive systemic organopathy, MRI, and catheter or MR angiogram, in order to exclude non-HIH. Hemorrhage due to cerebral amyloid angiopathy was not included in the HIH group. Hematoma was located in the thalamus in 9 patients, putamen in 3, pons in 2, and the lobar region in 3. Ventricular extension was found in 1 case and recurrent hemorrhage in another. Mean hematoma volume, calculated by the ABC/2 method [9] with a CT scan of ≤ 6 h from onset, was 17.2 cm^3 (range: 1.4–69.0, n = 15; a CT scan could not be obtained from 2 patients). None of these patients were treated with sympatomimetic agents or took illegal drugs. Aspirin was prescribed for an HIH patient with prior cerebral ischemic stroke. The group with ischemic stroke due to hypertensive vasculopathy included either atherothrombotic or lacunar infarction classified according to the criteria of National Institute of Neurological Disorders and Stroke [10]. The hypertension-only group was defined as having both a history of hypertension and no asymptomatic hemorrhages or infarcts on MRI. Neurological diagnoses of the hypertension-only group were as follows: spinocerebellar degeneration in 2, Creutzfeldt-Jakob disease in 2, transient neurological deficit in 2, unilateral metastatic brain tumor in 1, corticobasal degeneration in 1, multisystem atrophy in 1, progressive supranuclear palsy in 1, meningoencephalitis in 1, gait disturbance in 1, and headache in 1. All these disorders

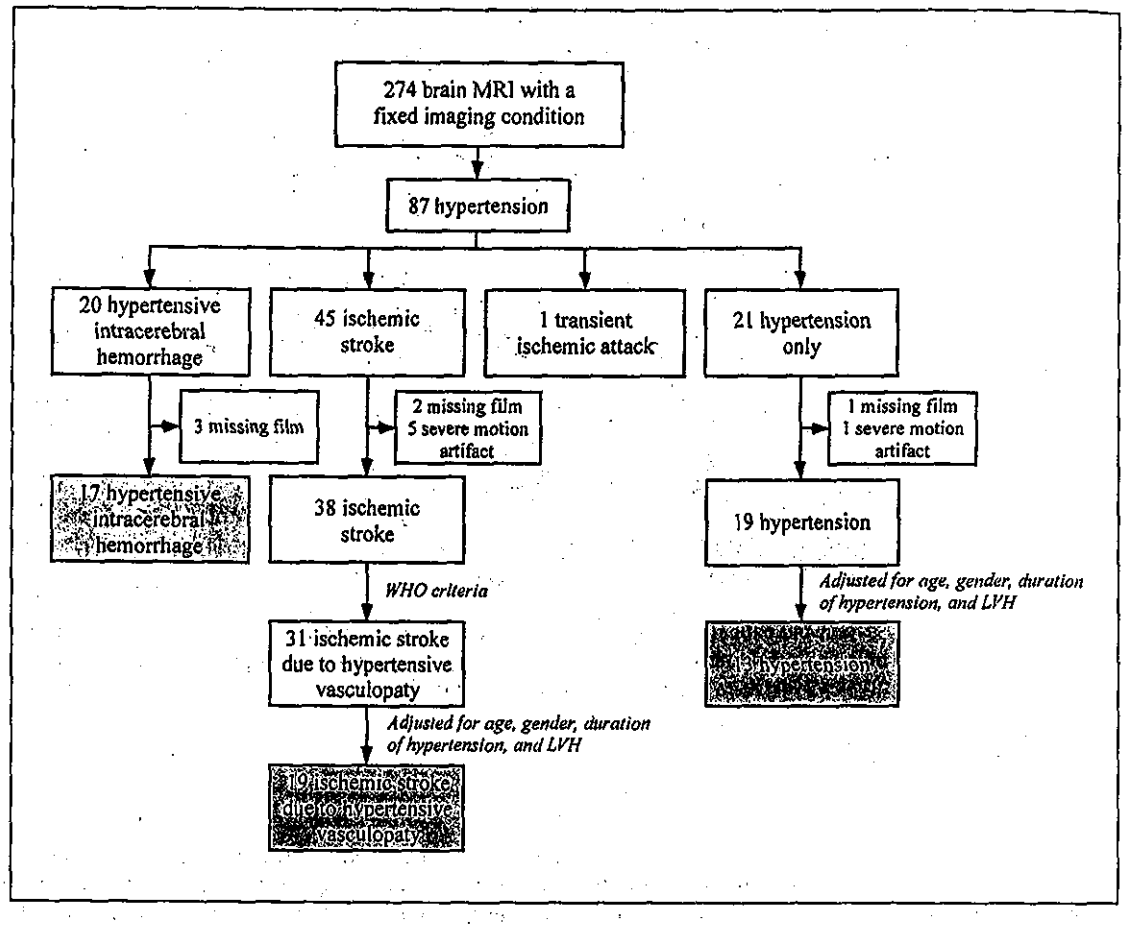

Fig. 2. Flow chart of subject selection. LVH stands for left ventricular hypertrophy.

were considered, at least at the time of the MRI examination, to have no influence on T_2-weighted MRI signals, in the cerebral hemisphere evaluated.

Definition of Vascular Risk Factors and MRI Condition

According to the JNC-7 report [11], hypertension was defined as a systolic blood pressure of ≥140 mm Hg or a diastolic blood pressure of ≥90 mm Hg based on the mean of 2 or more properly measured supine or seated blood pressure readings on 2 or more occasions. Upon reviewing clinical charts, both systolic and diastolic blood pressures were calculated as a mean value of at least 2 measurements on the day of the MRI examination for inpatients and during the last 2 weeks for outpatients. Diabetes mellitus was defined as having both symptoms of diabetes and a casual plasma glucose level of >11.1 mmol/l, a fasting plasma glucose level of >7.0 mmol/l, or a 2-hour plasma glucose level of >11.1 mmol/l during an oral glucose tolerance test [12]. Hypercholesterolemia was defined as >240 mg/dl total serum cholesterol and hypertriglycerolemia as >150 mg/dl fasting serum triglycerides. Smoking meant a history of smoking. Left ventricular hypertrophy was defined as either a cardiothoracic ratio of ≥50% on a chest X-ray, SV1 or SV2 + RV5 >3.5 mV on an electrocardiogram, or a posterior wall thickness of >12 mm on an echocardiogram. Serum creatinine was assessed in cases without nephropathy considered to result from nonhypertensive causes. All assessments were performed on each of the 49 patients.

MRI was performed using a 1.5-tesla superconductive scanner (Siemens Magnetom Vision, Erlangen, Germany) with a circular polarized head coil. Turbo spin-echo pulse sequences were routinely used to generate both T_1-weighted (a repetition time of 670.0 ms, an echo time of 14.0 ms) and T_2-weighted (a repetition time of 4,500.0 ms and an echo time of 96.0 ms) axial images of the brain. In each, the number of excitations was 2 and the scanning duration was 258 s. A matrix of 196 × 512 (phase × frequency) and a field of view of 173 × 230 mm were used. The slices were 5.0 mm thick and separated by a 1.0-mm interscan gap. Image reconstruction was carried out using two-dimensional Fourier transformation; smoothing was not performed.

Reliability Test and MRI Evaluation

To test the reliability of the manual measurement of LHOs, we prepared a card with a half-cut-out image of each patient at the uppermost corona radiata slice. We selected the side with fewer organic lesions (fig. 3). This way, any patient's identifying information including date, name, age as well as main organic lesion, was

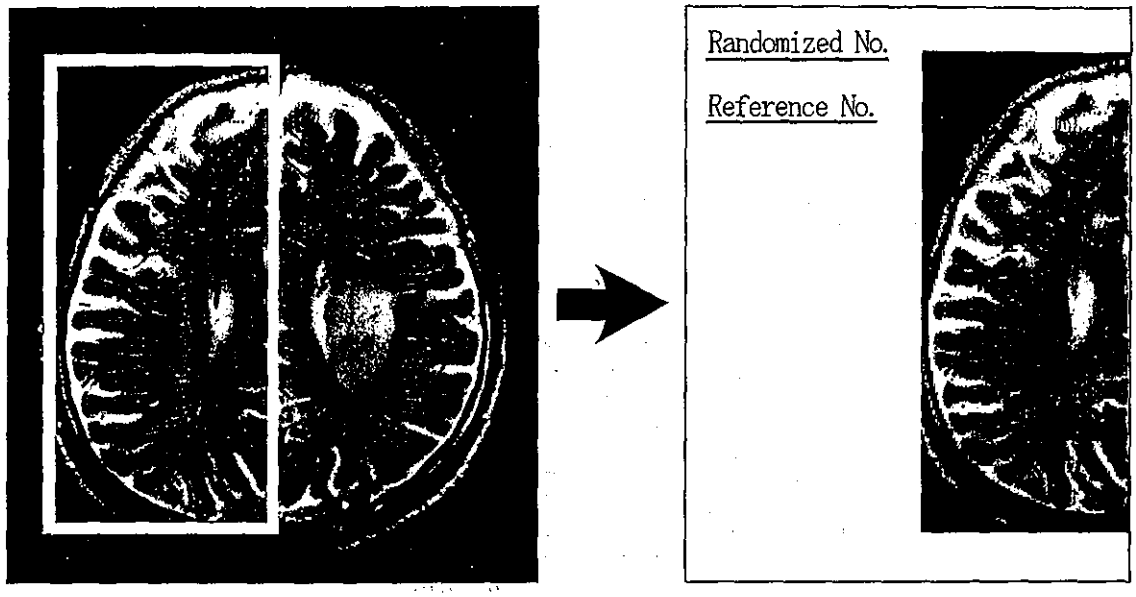

Fig. 3. *Schema of the half-cut-out image card for the reliability test.* We selected the uppermost corona radiata slice of the T_2-weighted MRI for each patient (left), and cut out the hemisphere with fewer organic lesions (right) in order to remove bias such as patient identifying information.

masked in the reliability test. Each card was presented to raters at random. At a point of two-thirds the distance from the lateral convexity to the lateral wall of the lateral ventricle, as we previously reported [5], we determined whether LHOs were present or absent, counted the number of LHOs with the naked eye, and measured the diameter of the LHOs with a 15× scale loupe. When a rater judged that the margin of the LHO diameter was unclear, it was excluded from the scaling. Therefore, in nearly all cases, the number of LHOs counted using a loupe was less than that of LHOs counted with the naked eye. The mean (SD) anterioposterior diameter ratio of the lateral ventricle to the brain on the slice studied was 0.35 (0.08) in the HIH, 0.33 (0.09) in the group with ischemic stroke due to hypertensive vasculopathy, and 0.32 (0.06) in hypertension-only group. Therefore, the measurement area of ischemic stroke due to hypertensive vasculopathy and hypertension-only was extended to a ratio of 0.35 (0.08) and 0.35 (0.04), respectively, to adjust to the HIH group. The measurement was taken from the right side in 10 patients, and from the left side in 7 patients in the HIH group, in 13 from the right side and in 6 from the left side in the group with ischemic stroke due to hypertensive vasculopathy, and in 6 from the right side and in 7 from the left side in the hypertension group. Using the half-cut-out images, an interrater test was performed by 4 independent raters (2 neurologists M.H. and S.T., and 2 stroke specialists K.M. and H.O.). An intrarater test was performed: one of the authors (M.H.) repeated the measurements four times in a 2-week period. Finally, presence of LHO, mean number, and mean diameter including the number of measurements were averaged by 4 raters for each patient.

Concerning other MRI findings, periventricular hyperintensities and subcortical white matter hyperintensities were scored as well based on Scheltens' scale (0–6 and 0–24, respectively) [13]. Microbleeds were defined on T_2-weighted MRIs as homogeneous, round, hypointense areas between 2 and 5 mm in diameter. Hypointense areas considered to be calcifications at the globus pallidus or a blood vessel were not regarded as microbleeds. Lacunar infarcts were defined as small, round, or oval-shaped lesions (<1.5 cm greatest diameter) [11], with both hypointensity on T_1-weighted and hyperintensity on a T_2-weighted MRI. The numbers of lacunar infarcts and microbleeds were counted on each slice. All MRI results were reviewed by an author (M.H.).

Statistical Methods

We compared the HIH group, the group with ischemic stroke due to hypertensive vasculopathy and the hypertension-only group using the Kruskal-Wallis H test with Scheffé's post hoc procedure, the Mann-Whitney U test, or the χ^2 test. Polynominal logistic regression analysis was performed to verify the independent explanatory variables for HIH by including all variables with $p < 0.05$ in the group comparison (current smoker, serum creatinine, antithrombotic medication, number of lacunar infarcts, microbleeds, and number and diameters of LHOs). Interrater and intrarater reliability was determined by calculating an intraclass correlation coefficient (ICC) through analysis of variance. This coefficient can be interpreted as a κ statistic: an ICC of 1.0 suggests perfect reliability, and an ICC >0.75 is generally considered to represent excellent reliability [14]. Finally, we evaluated each LHO number and diameter in relation to various clinical and radiological background factors using the Mann-Whitney U test or Spearman's rank test. Statistical significance was set at $p = 0.05$.

Table 1. Patients' characteristics

	HIH (n = 17)	Ischemic stroke due to hypertensive vasculopathy (n = 19)	Hypertension only (n = 13)	p value
Mean age, years (SD)	64.8 (10.3)	65.3 (6.7)	64.4 (10.8)	0.938
Male, n (%)	12 (70.6)	14 (73.7)	9 (69.2)	0.905
Mean systolic blood pressure, mm Hg (SD)	137 (15)	147 (20)	133 (12)	0.086
Mean diastolic blood pressure, mm Hg (SD)	86 (12)	85 (12)	82 (7)	0.394
Mean duration of hypertension, years (SD)	14.6 (9.1)	16.5 (8.5)	15.6 (11.3)	0.752
Antihypertensive medication, n (%)	13 (76.5)	12 (63.2)	3 (30.8)	0.062
Calcium channel blocker, n (%)	7 (41.1)	9 (47.4)	3 (23.1)	0.371
ACE inhibitor, n (%)	1 (5.9)	1 (5.3)	1 (7.7)	0.960
Controlled, n (%)	6 (35.3)	4 (21.1)	8 (61.5)	0.065
Diabetes mellitus, n (%)	6 (35.3)	12 (57.1)	2 (15.4)	0.059
Antidiabetic medication, n (%)	4 (23.5)	6 (31.5)	1 (7.7)	0.865
Controlled, n (% of diabetes)	3 (50.0)	3 (25.0)	1 (50.0)	0.598
Hypercholesterolemia, n (%)	6 (35.3)	6 (31.6)	4 (30.8)	0.959
Hypertriglycerolemia, n (%)	2 (11.8)	4 (21.1)	2 (15.3)	0.678
Statins, n (% of hyperlipidemia)	4 (66.7)	9 (100)	3 (75.0)	0.781
Controlled, n (% of hyperlipidemia)	3 (50.0)	3 (33.3)	3 (17.6)	0.234
Smoking, n (%)	9 (52.9)	10 (52.6)	5 (29.4)	0.919
Current smoker, n (%)	0 (0)	3 (15.8)	3 (17.6)	0.003
Left ventricular hypertrophy, n (%)	13 (76.5)	15 (79.0)	10 (76.9)	0.982
Serum creatinine, mg/dl (SD)	0.99 (0.25)	1.21 (0.43)[1]	0.89 (0.23)	0.044
Antithrombotic medication, n (%)	1 (5.9)	8 (42.1)	1 (7.7)	0.011

ACE = Angiotensin-converting enzyme.
[1] One case of glomerulonephritis was excluded.

Results

Clinical Background, Reliability Test, and MRI Results

Among the groups with HIH, ischemic stroke due to hypertensive vasculopathy and hypertension only, significant differences were found in current smokers ($p = 0.003$), patients with serum creatinine levels ($p = 0.044$), and patients on antithrombotic medication ($p = 0.011$) (table 1). Post hoc analysis showed significant differences in current smokers (HIH vs. ischemic stroke due to hypertensive vasculopathy; $p < 0.001$, and vs. hypertension only; $p = 0.001$) and the use of antithrombotic medication (HIH vs. ischemic stroke due to hypertensive vasculopathy; $p = 0.012$). No significant difference was found in serum creatinine by multiple comparisons.

Excellent reliability was identified for LHO numbers on interrater test ($\kappa = 0.92$, 95% CI = 0.88–0.95, SEM = 2.0%) and intrarater test ($\kappa = 0.95$, 95% CI = 0.92–0.97, SEM = 4.8%), LHO diameter on intrarater test ($\kappa = 0.89$, 95% CI = 0.83–0.93, SEM = 7.1%), and measured number of LHO diameters on interrater test ($\kappa = 0.87$, 95% CI = 0.80–0.92, SEM = 6.5%) and intrarater test ($\kappa = 0.94$, 95% CI = 0.90–0.96, SEM = 8.4%) tests. Interrater reliability of LHO diameters was low ($\kappa = 0.57$, 95% CI = 0.33–0.74, SEM = 33.1%).

In MRI results (table 2), no significant difference was found among the three groups in mean time from onset to MRI, severity of periventricular hyperintensities and subcortical white matter hyperintensities, and presence of LHO. There were significant differences in mean lacunar infarct number ($p < 0.0001$), in both the presence and number of microbleeds ($p = 0.015$ and 0.008, respectively), and the number of microbleeds at the basal ganglia or thalamus ($p = 0.007$). Post hoc analysis showed that compared with the hypertension-only group, the HIH group and the group with ischemic stroke due to hypertensive vasculopathy had a significantly higher mean lacunar infarct number ($p = 0.005$ and 0.001, respectively) and a higher presence of microbleeds ($p = 0.010$ and 0.012,

Table 2. MRI results

	HIH (n = 17)	Ischemic stroke due to hypertensive vasculopathy (n = 19)	Hypertension only (n = 13)	p value
Mean time from onset to MRI, days (range)	336.3 (12–1184)	203.8 (4–1417)		0.361*
Mean lacunar infarct number (range)	3.3 (0–12)	3.6 (0–10)	0	<0.0001
Periventricular hyperintensities				
Mean Scheltens's score (range)	1.7 (0–6)	1.7 (0–6)	1.2 (0–6)	0.556
Subcortical white matter hyperintensities				
Mean Scheltens's score (range)	9.7 (0–24)	9.1 (0–24)	5.0 (0–24)	0.769
Microbleeds				
Presence, %	70.6	68.4	23.1	0.015
Mean number (range)	3.0 (0–11)	2.9 (0–20)	0.4 (0–2)	0.008
Distribution, n (range)				
Lobar region	0.4 (0–2)	0.3 (0–4)	0.1 (0–1)	0.487
Basal ganglia/thalamus	2.0 (0–6)	1.8 (0–11)	0.2 (0–2)	0.007
Infratentorial	0.9 (0–4)	0.6 (0–5)	0.1 (0–1)	0.289
LHO				
Presence, %	100	100	100	
Mean number (SD)	10.7 (4.5)	7.0 (3.6)	5.5 (2.1)	0.002
Mean diameter, mm (SD)	0.67 (0.14)	0.59 (0.09)	0.56 (0.06)	0.007
Mean measured number (SD)	7.1 (3.0)	4.5 (2.2)	3.7 (1.1)	0.001

* Mann-Whitney U test between HIH and ischemic stroke due to hypertensive vasculopathy groups, excluding 2 cases of unclear onset in the ischemic stroke due to hypertensive vasculopathy.

respectively). No significant difference was found by multiple comparison in the total number of microbleeds and the number of microbleeds at the basal ganglia or thalamus. Concerning LHOs, there were significant differences in number, diameter, and measured number among the three groups (p = 0.002, 0.007, and 0.001, respectively). Post hoc analysis showed a significant difference in LHO number (HIH vs. ischemic stroke due to hypertensive vasculopathy; p = 0.013, and vs. hypertension only; p = 0.001, respectively), LHO diameter (HIH vs. hypertension only; p = 0.015), and the measured LHO number (HIH vs. ischemic stroke due to hypertensive vasculopathy; p = 0.008, and vs. hypertension only; p = 0.001, respectively).

Indicator of HIH and the Relationship to Each Background Factor

In polynomial logistic regression analysis, the LHO number was the most significant independent indicator of HIH (OR = 1.90, 95% CI = 1.16–3.12, p = 0.011) and the number of microbleeds was significant as well (OR 3.73, 95% CI = 1.10–12.65, p = 0.034). When LHO diameter was included in this model, it was the most significant independent indicator of HIH (OR per 0.1 mm: 1.76, 95% CI = 1.16–3.12, p = 0.011) followed by the number of microbleeds (OR = 2.90, 95% CI = 1.13–7.48, p = 0.028).

In categorical clinical or radiological variables, the LHO numbers of patients that took antihypertensive medication were significantly higher (p = 0.047) and the LHO diameters of current smokers were significantly lower (p = 0.016). No other significant difference was found. In continuous clinical or radiological variables, the LHO number and diameter did not significantly correlate with the time from onset to MRI in the HIH group (r = –0.24; p = 0.342, and r = 0.04; p = 0.868, respectively) and in the group with ischemic stroke due to hypertensive vasculopathy (r = 0.15; p = 0.523, and r = –0.05; p = 0.826, respectively), or with the size of hematomas (r = 0.18; p = 0.510, and r = 0.31; p = 0.263, respectively; n = 15). Diastolic blood pressure showed a significant correlation with LHO number among all patients (r = 0.29, p = 0.047). Other continuous values, including the degree of periventricular hyperintensities and subcortical white matter hyperintensities, lacunar infarct number, or number of microbleeds did not relate to either to LHO number or to diameter.

Discussion

This study showed with high reliability that LHO number is closely linked to the occurrence of HIH. Since linear hyperintensities in cerebral white matter on T_2-weighted MRIs are related to age [15–17] and severity of hypertension [5], we carefully adjusted age, gender, duration of hypertension and left ventricular hypertrophy of the group with ischemic stroke due to hypertensive vasculopathy and the hypertension-only group to those of the HIH group. We could not balance the serum creatinine level among groups due to the limited number of patients. The result was that the serum creatinine level tended to be higher in the group with ischemic stroke due to hypertensive vasculopathy. Although there was no a significant difference by multiple comparison, this fact is considered to reflect a common pathological basis in both lacunar infarct (the number of which was also higher in this group) and elevated serum creatinine [18]. In general, since both HIH and ischemic stroke due to hypertensive vasculopathy belong to stage III of the hypertension severity of WHO classification [8], it can be said that our study investigated the details of stage III.

Since HIH and lacunar infarction may share a common or related casual vessel lesion such as lipohyalinosis, depicted, in part, as microbleeds on T_2(*)-weighted MRIs [1, 19], it is difficult to predict whether HIH or ischemic stroke will occur in the most hypertensive patients, based on microbleeds. On the other hand, linear hyperintensities in the cerebral white matter on T_2-weighted MRIs were previously paid little attention from a clinical viewpoint. We have recently reported that LHOs increase in diameter according to the severity of hypertensive organopathy, and that they are probably based on arteriolar tortuosity of the white matter medullary artery and its dilated perivascular spaces [5, 6]. Overall, based on the results of our studies, prominent LHOs may reflect advanced microangiopathy, such as arteriolar tortuosity of the perforating arteries as well as that of the white matter medullary artery with an increased risk for bleeding, although we could not assess the former arterial territory using coronal or sagittal MRIs.

The actual source of bleeding in HIH has never been proven except for microaneurysm [20], but some reports have suggested the possibility of other mechanisms of vascular rupture in HIH [19, 21, 22]. Interestingly, Challa et al. [21] identified tortuous arterioles of the perforating arteries with an increased susceptibility of vascular rupture in HIH brains using high-resolution microangiography. Based on this report, while the relationship between white matter medullary arteries and perforating arteries remains unproven, prominent LHOs may reflect the cause of bleeding through a mechanism such as microdissection [21].

On the other hand, it may be said that, theoretically, LHOs reflect the epiphenomenon of HIH. From a pathological viewpoint, it has never been reported that hemorrhage affects the contralesional hemisphere except for mass effect. From the viewpoint of cerebral blood flow, it was shown that the autoregulation of global cerebral blood flow is constant in acute and treated stages [23]. Hyperperfused areas are sometimes found in the ipsilesional hemisphere in acute intracerebral hemorrhage, but never in the contralesional hemisphere [24–26]. From a clinical viewpoint, our results did not show any temporal relationship between the time from onset to MRI and LHOs, although the number of patients was small. Overall, it seems difficult to say that prominent LHOs result from intracerebral hemorrhage.

The interrater test of LHO diameter unexpectedly showed low reliability. This is likely due to the limitations of MRI resolution in manual measurements with a loupe. A smaller diameter of, at least, the y-axis direction of a pixel, a higher signal-to-noise ratio, and a shorter scan duration are imaging conditions to be considered for a future study, as well as an automatic measurement of LHOs such as segmentation. It has been unclear which factors related to high blood pressure cause LHOs, since the results of our study could not show any contributing factor. Since hemorrhagic stroke may occur in different types of blood pressure variation compared to ischemic stroke [27], it is considered that detailed clinical assessment including circadian blood pressure variation may be a key in unveiling the mechanism of LHO progression. Genetic susceptibility is also a considerable factor to be investigated [19]. Furthermore, the effect of antihypertensive treatment of LHOs has to be clarified in a long-term follow-up study.

In conclusion, the prominence of LHOs is closely linked to the occurrence of HIH. To clarify whether LHOs reflect the cause of and predict HIH, prospective, longitudinal studies are required using high-resolution MRI.

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Letter to the Editor

Association analysis between polymorphisms of the lymphotoxin-α gene and myocardial infarction in a Japanese population

Recently, a genome-wide association study revealed that variants in the lymphotoxin-α gene (*LTA*) are risk factors for myocardial infarction (MI), based on the multiplex PCR-Invader assay method at 92788 randomly selected gene-based SNPs [1]. It has also been shown that, in in vitro functional analyses, these variants might have some functional significance and that *LTA* may play a role in the pathogenesis of this disorder. However, association studies are plagued by the impression that they are not consistently reproducible [2,3]. Moreover, direct evidence of the contribution of *LTA* to atherogenesis is limited in both animals and humans [4]. Therefore, we performed an association analysis between polymorphisms of *LTA* and MI in a Japanese population.

Four hundred and seventy-seven male patients with MI (<70 years old) were recruited from the National Cardiovascular Center. The mean age was 56±8 years, with a range of 25–70 years. The control group consisted of 372 unrelated Japanese males <70 years old (mean age 59±9 years, range 30–70 years) recruited from the Suita study, which represents the general population in central Japan (Osaka) [5]. From the control group we excluded all subjects with a history of vascular diseases. Genomic DNA was isolated from leukocytes according to standard procedures. Polymorphisms were determined using the TaqMan system (PE Applied Biosystems). Three polymorphisms of *LTA*, *G10A* (exon1), *A252G* (intron1), and *C804A* (exon3), and one polymorphism of nuclear factor of κ light polypeptide gene enhancer in B cells, inhibitor-like 1 (*NFKBIL1*), and *T-63A* (promoter) were genotyped. All statistical analyses were performed with the JMP statistical package (SAS Institute Inc., USA).

The pattern of the frequency distribution of the genotypes is summarized in Table 1. These polymorphisms were almost completely concordant (i.e., the same allele frequencies and almost complete positive linkage disequilibrium). No significant deviation from Hardy–Weinberg equilibrium was observed. The −63A allele in *NFKBIL1* and the 10A, 252G, and A804 alleles in *LTA* were more common in patients than in controls. For example, in *LTA G10A*, a multiple logistic regression analysis, while adjusting for age and the prevalence

Table 1
Distribution of LTA genotypes in MI patients and controls

SNPs			Controls ($n = 372$)	MI patients ($n = 477$)	P
NFKBIL1 (A-63T)	Genotype	TT	166 (44.6%)	160 (33.6%)	0.004
		TA	157 (42.2%)	236 (49.6%)	
		AA	49 (13.2%)	80 (16.8%)	
	Allele frequency	T	0.66	0.58	0.002
		A	0.34	0.42	
LTA (G10A)	Genotype	GG	166 (44.7%)	160 (33.5%)	0.004
		GA	156 (42.1%)	235 (49.3%)	
		AA	49 (13.2%)	82 (17.2%)	
	Allele frequency	G	0.66	0.58	0.001
		A	0.34	0.42	
LTA (A252G)	Genotype	AA	163 (44.9%)	159 (33.4%)	0.003
		AG	153 (42.2%)	236 (49.6%)	
		GG	47 (13.0%)	81 (17.0%)	
	Allele frequency	A	0.66	0.58	0.001
		G	0.34	0.42	
LTA (A804C)	Genotype	CC	164 (44.8%)	161 (33.7%)	0.004
		CA	153 (41.8%)	236 (49.4%)	
		AA	49 (13.4%)	81 (17.0%)	
	Allele frequency	C	0.66	0.58	0.002
		A	0.34	0.42	

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of smoking, diabetes mellitus and hypercholesterolemia, revealed that the frequency of the A allele was significantly higher in patients with MI than in controls. An analysis which assumed that the A allele had dominant effects showed a significant association ($AA + AG$ versus GG: $P = 0.0025$, odds ratio 1.7, 95% CI 1.2–2.3). Although Ozaki et al. reported a significant association between the risk of MI and these polymorphisms, the distribution of genotypes was different (for example, in *LTA G10A*, GG/GA/AA (%): 39.4/49.1/11.5 in Control versus 36.7/44.5/18.8 in MI) and as a result, a recessive association model was assumed [1]. It is well known that one of the weaknesses of a case–control study is the selection of the control subjects, and this might explain the difference between the two studies [6].

Although the precise in vivo mechanism by which *LTA* influences the susceptibility to MI is unknown, the present study supports the notion that this gene is one of the most important genetic determinants of susceptibility to MI that has been detected so far.

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Potential of free-form TFPI and PAI-1 to be useful markers of early atherosclerosis in a Japanese general population (the Suita Study): association with the intimal-medial thickness of carotid arteries

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Abstract

This study assessed markers of vascular endothelial cell dysfunction associated with early atherosclerosis in carotid arteries. We measured the plasma levels of free-form tissue factor pathway inhibitor (free TFPI), plasminogen activator inhibitor-1 (PAI-1), and von Willebrand factor (vWF) in 522 adults without cardiovascular disease enrolled in the Suita Study. For each sex, we analyzed the association of the degree of intimal-medial thickness (IMT) with hemostatic markers using logistic regression analysis considering potential confounding risk factors, including age, body mass index, lifestyle (current smoking and drinking), illness (diabetes mellitus and hyperlipidemia), systolic blood pressure, and antihypertensive drug use. The age-adjusted levels of free TFPI and PAI-1 were positively and independently associated with the degree of IMT for men. Even after adjustment for all confounding factors, the level of PAI-1 was positively associated with the degree of IMT. These results indicate that measurement of the levels of free TFPI and PAI-1 is a potentially useful tool for the detection of early atherosclerosis in men.

Keywords: Atherosclerosis; Endothelium-derived factors; Hypertension

1. Introduction

Measurement of the intimal-medial thickness (IMT) of carotid arteries has been used as a non-invasive endpoint in epidemiological studies and clinical trials to assess the progression and regression of atherosclerosis [1,2]. Furthermore, IMT has recently been used not only as a surrogate endpoint for atherosclerosis of the coronary artery but also as a good indicator of the presence and extent of coronary artery disease [3–6]. Case-reference studies in a general population have been performed in regard to the association between markers of vascular endothelial cell dysfunction and atherosclerosis by measuring IMT of the carotid artery [7–9]. However, to detect early atherosclerosis, it is essential to study the association between these markers and the extent of atherosclerosis, using a general population free from cardiovascular disease (CVD).

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In this study, we focused on the association between three markers of endothelial cell dysfunction, namely free-form tissue factor pathway inhibitor (free TFPI), plasminogen activator inhibitor-1 (PAI-1), and von Willebrand factor (vWF) and IMT of carotid arteries in a Japanese general population (the Suita Study). Plasma concentrations of vWF and PAI-1 have previously been used as surrogate markers of endothelial damage [10,11]. TFPI inhibits tissue factor-initiated coagulation by binding to factor Xa and tissue factor-activated factor VII complex [12,13]. Most TFPI is synthesized by vascular endothelial cells, and is distributed into at least four pools in vivo. The majority of TFPI synthesized by vascular endothelial cells is associated with endothelial cells, whereas other pools circulate in the blood as complexes with lipoproteins (Lp-TFPI) or as a free form (free TFPI). A minor pool of TFPI is present in platelets. It has been demonstrated that free TFPI strongly correlates with endothelial cell markers such as thrombomodulin, vWF, and tissue-type plasminogen activator, whereas total TFPI does not [14]. There is a strong, positive correlation between the free TFPI and endothelial cell-associated TFPI levels [15]. Therefore, we selected free TFPI as a marker of endothelial cell dysfunction, instead of Lp-TFPI or total TFPI.

Here, we have demonstrated the potential of free TFPI and PAI-1 to be useful markers of early atherosclerosis by studying their association with IMT in relation to conventional risk factors for CVD.

2. Methods

2.1. Study population

The study population was based on samples randomly selected from the residents of Suita, a city located in the second largest urban area in Japan (the Suita Study) [5]. The subjects have been visiting the National Cardiovascular Center every 2 years since 1989 for regular health checkups. Only subjects who provided written informed consent to have a blood examination were enrolled in this study. The subjects included 245 men and 277 women who were free of cardiovascular disease, aged from 34 to 91 years, and attended the National Cardiovascular Center from 5 August 1998 to 24 December 1998. Subjects were classified as smokers if they smoked at least one cigarette per day. Subjects were defined as hypertensive if their diastolic blood pressure was $\geq$95 mmHg, their systolic blood pressure was $\geq$160 mmHg, or they were taking antihypertensive medication. Subjects whose fasting blood glucose levels were $\geq$7.78 mmol/L, whose blood glucose levels were $\geq$11.11 mmol/L 2 h after a 75-g oral glucose loading, or who were taking antidiabetic medication were defined as diabetic. Subjects whose total serum cholesterol level was $\geq$5.68 nmol/L (220 mg/dl), or who were taking anti-hypercholesterolemic medication were defined as having hypercholesterolemia.

2.2. IMT measurements

The details of the ultrasonic carotid examination have previously been published [16]. We used a high-resolution B-mode ultrasonic machine with 7.5-MHz transducers, yielding an axial resolution of 0.2 mm. The regions between 30 mm proximal from the beginning of the dilation of the bifurcation bulb and 15 mm distal from the flow divider of both common carotid arteries were scanned. All measurements were made at the time of scanning using the instrument's electronic caliper and were recorded as photocopies. The IMT in common carotid arteries was measured on a longitudinal scan of the common carotid arteries at a point 10 mm proximal from the beginning of the dilation of the bifurcation bulb. We defined the IMT as mean IMT of the near and far walls at the point of measurement.

2.3. Blood collection and analysis

After a minimum 12-h fast and between 10 a.m. and 1 p.m., blood samples for hemostatic profile were collected into disposable, siliconized, evacuated glass tubes containing 0.1 vol. of 3.13% trisodium citrate, and blood collected in a second tube was used for the coagulation assay. The samples were centrifuged at 4600 $\times$ g for 10 min at room temperature within 1 h of collection. The PAI-1 antigen level was immediately determined, and the remaining plasma was aliquoted in plastic tubes and stored at $-80\,^{\circ}C$ until use. The thawed samples were used to measure free TFPI and vWF.

The antigen level of free TFPI was measured by a sandwich enzyme immunoassay method [17]. The coefficient of intra-assay variation for the assay was 2.7%. The antigen levels of PAI-1 and vWF were automatically measured by latex photometric immunoassay using an LPIA-tPAI kit (Mitsubishi Kagaku Medical) and STA liatest vWF kit (Diagnostica Stago), respectively. The coefficients of intra-assay variation of PAI-1 and vWF were 1.0 and 4.3%, respectively.

2.4. Statistical analysis

All statistical analyses were performed independently by sex. We first used Spearman correlation analysis to assess the association between the progression of IMT and the analyzed parameters (Tables 1 and 2). We then used ANCOVA to investigate whether plasma levels of free TFPI, PAI-1, and vWF are positively and independently associated with the degree of carotid intimal thickness or not (Table 3). We have performed two types of adjustments. First, adjustments were made for age only. Second, further adjustments were made for lifestyle (drinking and smoking), illness (diabetes, hypercholesterolemia), body mass index, systolic blood pressure, and antihypertensive drug use. Differences with a value of $P < 0.05$ for ANCOVA were

Table 1
Demographic characteristics and unadjusted hemostatic parameters according to rank of intimal-medial thickness (IMT) of the carotid artery in men

	IMT-rank				P
	Q1 ($n = 58$)	Q2 ($n = 70$)	Q3 ($n = 57$)	Q4 ($n = 60$)	
Median of IMT (mm)	0.73	0.83	0.93	1.05	
Age (year)	47.4 ± 7.8	59.2 ± 9.2	69.2 ± 8.7	70.4 ± 8.0	<0.0001
Current drinking (%)	79	73	67	62	<0.0009
Smoker (%)	78	73	67	62	<0.0007
Body mass index (kg/m^2)	23.2 ± 3.3	23.7 ± 3.2	23.4 ± 3.3	22.8 ± 3.1	<0.4217
Diabetes (%)	0	3	5	10	<0.0511
Hypertension (%)	7	29	32	45	<0.0001
Hypercholesterolemia (%)	7	11	9	15	<0.1685
LDL-cholesterol (mg/dl)	113.9 ± 27.5	124.8 ± 29.5	120.3 ± 26.7	131.0 ± 26.4	<0.0060
HDL-cholesterol (mg/dl)	60.8 ± 17.2	56.1 ± 12.5	55.2 ± 16.1	55.2 ± 16.1	<0.0255
Free TFPI (ng/ml)	15.7 ± 4.5	16.0 ± 3.8	17.2 ± 3.2	18.2 ± 4.6	<0.0006
PAI-1 (ng/ml)	32.3 ± 25.2	32.9 ± 30.9	29.3 ± 19.7	33.0 ± 38.8	<0.2059
von Willebrand factor (%)	115.0 ± 43.7	137.1 ± 45.9	151.7 ± 54.9	160.8 ± 63.9	<0.0001

Values are mean ± S.D. or percent. *P*-values were calculated by simple linear regression analysis. TFPI; tissue factor pathway inhibitor, PAI-1; plasminogen activator inhibitor-1.

Table 2
Demographic characteristics and unadjusted hemostatic parameters according to rank of intimal-medial thickness (IMT) of the carotid artery in women

	IMT-rank				P
	Q1 ($n = 66$)	Q2 ($n = 73$)	Q3 ($n = 63$)	Q4 ($n = 75$)	
Median of IMT (mm)	0.70	0.78	0.85	0.95	
Age (year)	45.8 ± 6.7	55.2 ± 7.6	62.2 ± 7.2	71.9 ± 7.8	<0.0001
Current drinking (%)	45	33	35	25	<0.0897
Smoker (%)	12	5	5	8	<0.6107
Body mass index (kg/m^2)	21.3 ± 2.5	21.6 ± 2.9	23.2 ± 3.5	22.9 ± 3.6	<0.0145
Diabetes (%)	2	0	5	4	<0.6649
Hypertension (%)	2	10	19	40	<0.0001
Hypercholesterolemia (%)	8	10	11	19	<0.0913
LDL-cholesterol (mg/dl)	115.4 ± 30.5	126.4 ± 30.0	137.9 ± 25.7	137.0 ± 26.5	<0.0001
HDL-cholesterol (mg/dl)	73.1 ± 17.9	69.2 ± 15.5	67.5 ± 16.7	62.1 ± 14.8	<0.0002
Free TFPI (ng/ml)	11.5 ± 3.2	14.9 ± 5.0	16.3 ± 4.2	17.5 ± 4.8	<0.0001
PAI-1 (ng/ml)	20.2 ± 18.3	19.8 ± 12.8	25.7 ± 19.1	26.2 ± 18.0	<0.0707
von Willebrand factor (%)	107.8 ± 31.4	126.5 ± 41.8	136.5 ± 51.6	157.2 ± 59.2	<0.0001

Values are mean ± S.D. or percent. *P*-values were calculated by simple linear regression analysis. TFPI; tissue factor pathway inhibitor, PAI-1; plasminogen activator inhibitor-1.

considered to be significant. All analyses were performed with SAS statistical software (release 8.2 SAS Institute Inc).

3. Results

3.1. Demographic characteristics and unadjusted parameters according to rank of IMT of carotid arteries

We measured IMT in a general population, divided it into four quartiles by sex, and analyzed the demographic characteristics and unadjusted parameters according to IMT rank, as shown in Tables 1 and 2. The IMT median of each quartile (*Q*1, *Q*2, *Q*3 and *Q*4) is shown in the first column of each table. In both sexes, the plasma levels of free TFPI, vWF, and LDL-cholesterol as well as age and hypertension increased in a stepwise manner from the first to the forth IMT quartile.

3.2. Multivariate analysis of free TFPI, PAI-1, and vWF levels according to IMT rank

As summarized in Table 3, we analyzed the plasma levels of free TFPI, PAI-1, and vWF according to IMT ranks after either adjusting for age only or adjusting for age, lifestyle (drinking and smoking), body mass index, systolic blood pressure, diabetes, hypercholesterolemia, and hypertensive drug use. Age adjusted free TFPI levels in men increased in a stepwise manner from the first to the fourth IMT quartile ($P = 0.003$, for trend) and the levels of free TFPI in the third and the fourth quartiles compared to the lowest IMT quartile remained statistically significant in the multivariate analysis. However, the statistically significant increases of free TFPI

Table 3
Adjusted mean levels of free TFPI, PAI-1, and von Willebrand factor according to rank of intimal-medial thickness (IMT) of the carotid artery

	IMT-rank				*P* for trend
	Q1	*Q2*	*Q3*	*Q4*	
Free TFPI (ng/ml)					
Men					
Age adjusted	15.3 ± 0.7	15.9 ± 0.5	17.5 ± 0.5‡	18.4 ± 0.6‡	0.003
All adjusted	16.2 ± 0.7	15.9 ± 0.5	17.2 ± 0.6	17.9 ± 0.6	0.075
Women					
Age adjusted	14.3 ± 0.7	15.8 ± 0.5‡	15.6 ± 0.5	14.8 ± 0.6	0.410
All adjusted	14.9 ± 0.6	15.9 ± 0.5	15.2 ± 0.5	14.5 ± 0.6	0.250
PAI-1 (ng/ml)					
Men					
Age adjusted	21.9 ± 4.9	31.1 ± 3.5	35.1 ± 4.2	39.8 ± 4.3‡	<0.001
All adjusted	24.1 ± 5.3	30.8 ± 3.5	34.7 ± 4.3	38.3 ± 4.5	<0.001
Women					
Age adjusted	18.0 ± 2.9	19.1 ± 2.1	26.3 ± 2.2‡	28.4 ± 2.7‡	0.227
All adjusted	20.3 ± 2.6	20.8 ± 1.9	23.7 ± 2.0	26.9 ± 2.5	0.317
von Willebrand factor (%)					
Men					
Age adjusted	144.0 ± 8.3	142.1 ± 6.0	135.6 ± 7.2	143.0 ± 7.3	0.353
All adjusted	141.3 ± 8.9	142.1 ± 6.0	136.9 ± 7.3	144.4 ± 7.7	0.180
Women					
Age adjusted	133.7 ± 7.6	134.0 ± 5.5	130.8 ± 5.9	131.8 ± 7.1	0.042
All adjusted	133.5 ± 7.7	134.7 ± 5.7	129.9 ± 6.0	132.0 ± 7.4	0.049

Values are mean ± errors adjusted for age or adjusted for age, life style (current drinking and smoking), body mass index, present illness (diabetes, hypercholesterolemia), systolic blood pressure, and hypertensive drug use. (‡) $P < 0.05$ compared with Q1 subjects. TFPI; tissue factor pathway inhibitor, PAI-1; plasminogen activator inhibitor-1.

levels in men were not detected after adjustment for several possible confounding factors (all adjusted). The free TFPI levels in women demonstrated a mountain-shaped relationship with the degree of IMT in the multivariate analysis.

Age-adjusted PAI-1 levels in men increased with increasing IMT rank ($P < 0.001$) and the levels in the fourth quartile compared to the lowest IMT quartile remained statistically significant in the multivariate analysis. Age-adjusted PAI-1 levels in women also increased with increasing IMT rank and the levels in the third and fourth quartiles compared to the lowest quartile were statistically significant, although the *P* value for trend was 0.227. The statistically significant increases of PAI-1 levels in men was detected after all adjustments.

In contrast, the age-adjusted vWF levels in both sexes did not show significant changes among IMT quartiles, although the *P* values for trend in women after all adjustments were significant. These results indicate that measurement of the levels of free TFPI and PAI-1 is a potentially useful tool for the detection of early atherosclerosis in men.

4. Discussion

In this cross-sectional analysis, we have demonstrated that increased levels of free TFPI and PAI-1 in men without CVD were closely associated with the elevation of IMT in common carotid arteries as measured by B-mode ultrasonography. These findings suggest that free TFPI and PAI-1 may be sensitive markers reflecting early atherosclerosis in the carotid arteries.

It has been demonstrated that TFPI localizes with tissue factor within atherosclerotic plaques in human carotid and coronary arteries and modulates the thrombogenicity of the plaque by attenuating the tissue factor activity [18–20]. Enhancement of TFPI expression in the atherosclerotic plaque will cause an increase in the free TFPI concentration in the plasma of patients with cardiovascular disease. In fact, elevated free TFPI levels have been reported in the plasma of patients with ischemic heart disease [21,22]. These findings imply that an elevated level of free TFPI in the plasma is closely associated with hypercoagulable states in atherosclerotic diseases. However, the role of TFPI associated with subclinical or early atherosclerosis was rarely reported. Sakkinen et al. reported a significant positive relationship between the level of plasma TFPI activity and subclinical cardiovascular disease in a healthy elderly cohort study [23]. However, the relationship between age/gender and TFPI levels in a general population has not been examined in detail. In this study, we have extended the above results and demonstrated a direct link between the extent of carotid artery atherosclerosis and the plasma level of free TFPI antigen in men in a Japanese general population without CVD.

As summarized in review articles [24,25], many investigators have demonstrated the crucial role of PAI-1 in human atherothrombosis. High plasma PAI-1 concentrations are associated with various thrombotic diseases and are independent risk factors for myocardial infarction, as has been proven by epidemiological studies. Animal experiments using PAI-1 transgenic mice and PAI-1 knock-out mice also support the role of PAI-1 in the progression of atherosclerosis [26–28]. These previous findings suggest that an increased PAI-1 level in the plasma is closely associated with the progression of atherosclerotic conditions. Here, we have demonstrated that this relationship could also be observed in early atherosclerotic conditions in a general population.

We have previously reported that the mean IMT value of both sexes increased stepwise with the number of major coronary risk factors, namely hypertension, smoking, and hypercholesterolemia [5]. It has also been reported that PAI-1 levels were increased in patients with early hypertension, and that these elevated PAI-1 levels were improved by treatment with angiotensin-converting enzyme inhibitor [29]. Recently, it has been reported that PAI-1 deficiency prevents hypertension and vascular fibrosis in response to long-term nitric oxide synthase inhibition [30]. Taken together, these data indicate that early atherosclerotic conditions or endothelial cell dysfunction are induced by hypertensive conditions, resulting in elevation of the plasma levels of free TFPI and PAI-1. Therefore, it is thought that the close association between elevation of the plasma levels of free TFPI and PAI-1 and hypertensive conditions probably diminished the statistically independent association between plasma levels of free TFPI and PAI-1 and the degree of IMT.

In conclusion, we have demonstrated that the levels of free TFPI and PAI-1 in men increased with the degree of IMT. Therefore, we propose that free TFPI and PAI-1 are potentially useful markers for detecting early atherosclerosis. However, prospective studies are necessary to clarify whether these markers are predictive of the onset of atherosclerotic diseases in Japanese people.

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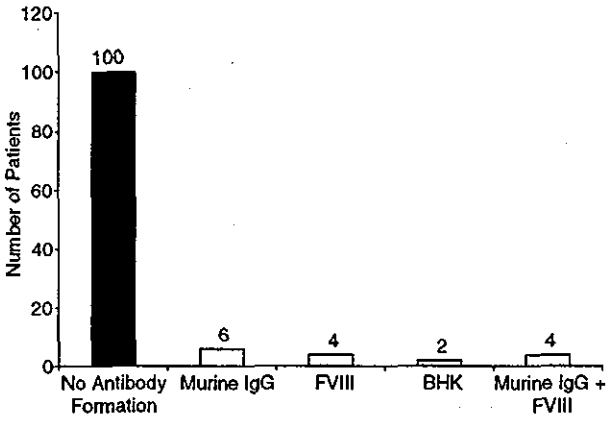

Fig. 1. Antibody development to trace mammalian proteins ($n = 116$ patients).

Nineteen of 100 (19%) antibody-negative patients and seven of 16 (44%) antibody-positive patients had adverse events with an allergic/hypersensitivity component at sometime during the study. Five of 100 antibody-negative patients experienced drug-related adverse events with an allergic/hypersensitivity component; however, none of the 16 antibody positive patients experienced such drug-related adverse events. Antibody responses were not associated temporally with allergic or hypersensitivity drug-related adverse events. Ingerslev *et al.* examined serum samples collected from PTPs and PUPs treated with Recombinate™ for antibodies to trace heterologous proteins [1]. These investigators reported antibody reactivity following treatment with rFVIII to Chinese hamster ovary protein, bovine serum albumin, and murine IgG in PUPs (25%, 37%, 41%, respectively) and PTPs (4.4%, 8%, and 7%, respectively). As in the current study, these investigators found no association between the development of these antibodies and adverse events.

These data indicate that a small number of naive patients treated with a recombinant preparation may be susceptible to development of antibodies to residual trace proteins, but these antibodies have no clinical effect. Patients with inhibitors may have an increased propensity to develop antibodies in general, as they appeared to have a higher incidence of antibody development to trace mammalian proteins than did patients that did not develop an inhibitor on study.

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Prevalence of protein S deficiency in the Japanese general population: The Suita Study

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Protein S is a vitamin K-dependent anticoagulant protein that serves as a cofactor for activated protein C. Protein S deficiency is a well-known risk factor for venous thrombosis [1]. However, the prevalence of protein S deficiency in the population has not been determined. Recently, using 3788 healthy blood donors in the West of Scotland, the prevalence of protein S deficiency was reported to be between 0.03% and 0.13% [2]. In a Japanese population, a relatively high prevalence of three heterozygotes for the Lys155Glu substitution in the second epidermal growth factor (EGF)-like domain of protein S molecule for every 182 Japanese, has been reported [3]. Subsequent functional analysis of this mutant protein S indicated that it possessed low cofactor activity for activated protein C, suggesting that the Japanese population carries this mutant at significant levels, thereby conferring an increased risk of thrombosis [4]. We therefore undertook a population-based study to determine the prevalence of protein S deficiency in the Japanese.

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We used a randomly selected population from the residents of Suita, a city located in the second largest urban area in Osaka, Japan [5]. In the present study, 2690 consecutive blood donors (1252 males and 1438 females) aged 33–89 years were enrolled for measurement of protein S and antithrombin activities. Protein S activity was measured as cofactor activity for activated protein C on the basis of the activated partial thromboplastin time assay using Staclot protein S (Diagnostica Stago, Asnières, France). Antithrombin activity was measured as a heparin cofactor activity using a chromogenic substrate S-2238 (Chromogenix AB, Stockholm, Sweden).

The mean (SD) activity of protein S in males and females was 92.6% (21.4%) and 82.9% (17.8%), respectively ($P < 0.0001$), indicating that males have higher protein S activity than females. These gender differences were observed in all age ranges, except for 70 and 80 years of age. Age-related changes were observed in males (correlation coefficient, $r = -0.366$), but not in females. Therefore, we tried to determine the prevalence of protein S deficiency by sex.

In identifying plasminogen deficiency, we previously adopted combined criteria of plasminogen activity below mean − 2 SD and a ratio of plasminogen activity to antithrombin activity over mean + 2 SD [6]. Using the same criteria, we considered individuals with protein S activity below mean − 2 SD (male < 49.8%, female < 47.4%) and an antithrombin activity/protein S activity ratio over mean + 2 SD (male > 1.72, female > 1.98) as having protein S deficiency. As a result, 14 males and 23 females were identified as protein S deficient. Therefore, the prevalence of protein S deficiency was estimated to be 1.12% in males and 1.60% in females. The prevalence of protein S deficiency in females might have been overestimated due to the effects of hormonal state [7], so we considered 1.12% to represent the prevalence of protein S deficiency in the Japanese general population.

Our results in this study agree with the previously reported prevalence of 2%, obtained from 392 healthy Japanese [8]. However, they were not consistent with results obtained from a Caucasian population (0.03–0.13%) [2]. This difference probably derives from the different racial backgrounds of the two populations or the use of different assay methods. As described, the Japanese population carries the Lys155Glu substitution in the protein S molecule that decreases functional activity [3,4]. Genotype–phenotype correlation of functional protein S in the Japanese population remains to be done.

We previously reported that the prevalence of protein C and antithrombin deficiency in the Japanese general population was estimated to be 0.13% and 0.15%, respectively [9]. We also reported plasminogen deficiency to be 4.3% [6]. The population utilized for these studies was the same as in the present study. Therefore, we conclude that the prevalence of protein S deficiency in Japanese is much higher than that of protein C and antithrombin deficiency and lower than that of plasminogen deficiency.

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Recent Advances in Thrombotic Thrombocytopenic Purpura

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Thrombotic thrombocytopenic purpura (TTP) is characterized by microangiopathic hemolytic anemia and thrombocytopenia, accompanied by microvascular thrombosis that causes variable degrees of tissue ischemia and infarction. Intravascular coagulation is not a prominent feature of the disorder. Plasma exchange can induce remissions in approximately 80% of patients with idiopathic TTP, but patients have a much worse prognosis when thrombotic microangiopathy is associated with cancer, certain drugs, infections, or tissue transplantation. Recently, acquired autoimmune deficiency of a plasma metalloprotease named ADAMTS13 was shown to cause many cases of idiopathic TTP. This review describes our current understanding of how to use this knowledge clinically.

In Section I, Dr. Joel Moake describes the presentation of thrombotic microangiopathy, emphasizing the pathophysiology of idiopathic TTP. Platelets adhere to ultra-large (or "unusually large") von Willebrand factor (ULVWF) multimers that are immobilized in exposed subendothelial connective tissue and secreted into the circulation in long "strings" from stimulated endothelial cells. ADAMTS13 cleaves ULVWF multimers within growing platelet aggregates under flowing conditions, and this normally limits platelet thrombus formation. If ADAMTS13 is absent, either congenitally or due to acquired autoantibodies, platelet-rich microvascular thrombosis proceeds unchecked and TTP ensues. Plasma exchange is effective therapy for idiopathic TTP, probably because it replenishes the deficient ADAMTS13 and removes some of the pathogenic autoantibodies and endothelial-stimulating cytokines. Some patients have a type of thrombotic microangiopathy after transplantation/chemotherapy but do not have severe ADAMTS13 deficiency. The pathogenesis of their disease must differ but remains poorly understood.

In Section II, Dr. Toshiyuki Miyata describes recent advances in assay methods that should facilitate routine laboratory testing of ADAMTS13 for patients with thrombotic microangiopathy. ADAMTS13 cleaves a single Tyr-Met bond in domain A2 of the VWF subunit. ADAMTS13 assays based on the cleavage of plasma VWF multimers have been used extensively but require considerable time and expertise to perform. A recombinant substrate containing 73 amino acid residues of VWF domain A2 has been devised that allows short incubation times and rapid product detection by gel electrophoresis or immunoassay. These results should encourage the development of even simpler assays that can be performed in most clinical laboratories.

In Section III, Dr. James George provides an update on the long-term prospective study of thrombotic microangiopathy in the Oklahoma TTP-HUS Registry. At presentation, the clinical distinction between idiopathic TTP, various forms of secondary thrombotic microangiopathy, and even Shiga toxin–associated hemolytic uremic syndrome (HUS) can be problematic because the symptoms and laboratory findings often overlap. Consequently, plasma exchange usually is administered to any patient with thrombotic microangiopathy if there is doubt about the cause. The role of ADAMTS13 testing in choosing therapy remains uncertain, but the results do appear to have prognostic significance. Severe ADAMTS13 deficiency is specific for idiopathic TTP and identifies a subgroup with a high likelihood of response to plasma exchange, and high-titer ADAMTS13 inhibitors correlate strongly with a high risk of relapsing disease. Patients with normal ADAMTS13 activity have a much worse prognosis, although many factors probably contribute to this difference. Longitudinal study of these patients will continue to clarify the relationship of ADAMTS13 deficiency to the clinical course of thrombotic microangiopathy.