Additional impact of morning haemostatic risk factors and morning blood pressure surge on stroke risk in older Japanese hypertensive patients

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Received 27 July 2010; revised 5 October 2010; accepted 27 October 2010; online publish-ahead-of-print 18 December 2010

Aims
Stroke events occur most frequently in the morning hours. Impaired haemostatic activity and morning blood pressure (BP) surge, defined as the morning BP increase from sleep, have individually been associated with stroke risk in general or hypertensive populations. However, their combined impact on the risk of a stroke remains unknown.

Methods and results
A total of 514 hypertensive patients aged >50 years (mean 72.3 years; 37% men) underwent 24 h BP monitoring, measurement of haemostatic risk factors [plasma fibrinogen, plasminogen activator inhibitor-1 (PAI-1), and prothrombin fragment 1+2 (F1+2)], and brain MRI at baseline. The incidence of stroke was prospectively ascertained. During an average of 41 months (1751 person-years), there were 43 stroke events (ischaemic, 30; haemorrhagic, 5; undefined, 8). On multivariable analysis adjusted for confounding factors, the hazard ratio [HR (95% confidence interval (CI))] for stroke in the highest vs. lower quartiles of PAI-1 was 2.5 (1.3–4.6), that for F1+2 was 2.6 (1.4–5.0), and that for the morning BP surge was 1.2 (1.1–1.4; all P < 0.01). In particular, the ratio was substantially higher in cases with the highest quartile of both PAI-1 and F1+2 levels compared with those with the lower quartiles of both parameters (HR: 8.2; 95% CI: 3.7–18.2; P < 0.001). Among the patients with the highest quartile of the morning BP surge (n = 128), the multivariable HR (95% CI) for the highest vs. lower quartiles of PAI-1 was 3.4 (1.3–9.1) and that for F1+2 was 3.3 (1.3–8.7) (both P < 0.05).

Conclusion
High levels of plasma PAI-1 and F1+2, as well as an excessive morning BP surge, are independently and additively associated with an increased risk of stroke in older hypertensive patients.

Keywords
Plasminogen activator inhibitor-1 • Prothrombin fragment 1+2 • Fibrinogen • Morning blood pressure surge • Stroke

Introduction
The incidence of cardiovascular events, such as myocardial infarction, sudden death, and stroke, is highest in the early hours after waking.1,2 The mechanisms involved in the morning increase in cardiovascular events have been unclear, but recent clinical studies have shown that an exaggerated morning blood pressure (BP) surge is a plausible factor involved in the triggering of cardiovascular events, particularly in the case of stroke.3–5

Haemostatic risk factors are associated with an increased risk of stroke.5–9 Physiologically, haemostatic activity is exaggerated in the morning; i.e. hypercoagulable and hypofibrinolytic states occur in the morning.10,11 Therefore, exaggerated morning impairment of haemostatic activity may also contribute to the morning increase in stroke events.1,2,12

Exaggerated morning BP surge has been closely associated with neurohumoral risk factors potentiated in the morning [e.g. sympathetic nerve activity (SNA) and the renin-angiotensin-aldosterone
system (RAAS); these neurohumoral risk factors also affect haemo-
static activity [e.g. SNA–platelet interaction and RAAS–plasmino-
gen activator inhibitor-1 (PAI-1) interaction].2,13–15 Therefore, the
morning BP surge and haemostatic risk factors may be associated
with each other and may have an additional impact on stroke
events in hypertensive patients. However, no study has investigated
the impact of haemostatic risk factors on stroke incidence in relation
to the morning BP surge.

In the present study, therefore, we examined whether or not
haemostatic risk factors are associated with clinical stroke events
as well as silent cerebral infarction (SCI: a predisposing condition
of stroke) in relation to the presence of the morning BP surge in
stroke-prone Japanese older hypertensive patients.

Methods

Subjects

The methods of the study have been detailed elsewhere.3,16 Briefly,
we initially enrolled 821 older hypertensive outpatients (clinic BP
≥140/90 mmHg and age ≥50 years) in the Jichi Medical School
ambulatory blood pressure monitoring (JMS-ABPM) study, wave 1,
from six participating institutions (three clinics, two hospitals, and
one outpatient clinic of a medical school) between 1 January 1992
and 1 January 1998. The patients who had a history of stroke, coronary
artery disease (CAD), chronic heart failure, peripheral vascular
disease, atrial fibrillation, and obvious present illness (e.g. malignancy
or infection) at baseline were excluded from this study. Follow-up
examination was successfully conducted in 810 (99%) patients.
Among them, analysis was performed for 514 patients after we
excluded the patients who did not agree to undergo brain MRI
(296 patients) or with incomplete data (one patient) at baseline.
There were no significant differences in the patients’ characteristics
and baseline blood sampling data between the patients who under-
went brain MRI and those who did not.

Clinic BP was measured after at least 5 min of seated rest. Dia-
abetes mellitus was defined as a fasting glucose level ≥126 mg/dL,
a random non-fasting glucose level ≥200 mg/dL, haemoglobin
A1c ≥6.2%, or the use of an oral hypoglycaemic agent or insulin.
Hyperlipidaemia was defined as a total cholesterol level
≥240 mg/dL or the use of an oral lipid-lowering agent. Smokers
were defined as current smokers or not. This study was approved
by the independent research Ethics Committee, Jichi Medical
University School of Medicine, Japan, in 1998. All of the subjects
studied were ambulatory and all gave informed consent to partici-
pate in the study. Some of the data from the JMS-ABPM study
were published previously.3,16,17 This article is a subanalysis of the
JMS-ABPM study.

Twenty-four hour ambulatory blood pressure
monitoring and brain magnetic resonance imaging

The methods used for the 24 h ABPM3,16,17 are detailed in Supplemen-
tary material online. The subjects stopped antihypertensive drugs for at
least 14 days before the ABPM study (n = 285, 55%). The morning BP
surge was calculated as follows: the average of the BP readings over the
2 h after waking up minus the average BP from the three readings
centred on the lowest night-time reading.3

On brain MRI, an SCI was defined as a low signal intensity area (3–
15 mm) on T1-weighted images that was also visible as a hyperintense
lesion on T2-weighted images, as described previously.3,18,19 The MRI
images of the subjects were randomly stored and interpreted by two
neurologists who were blind to the subjects’ names and characteristics,
and the reproducibility of the MRI reading was demonstrated previously.3,16,17

Assay for haemostatic risk factors

Venous blood was obtained in the morning (8 a.m. – 10 a.m.) after
an overnight fast. Blood samples to assess haemostatic risk factors were
collected after 10 min in the supine position by the two-syringe
method into disposable siliconized vacuum glass tubes containing a
1/10 volume of 3.8% trisodium citrate. The plasma fibrinogen level
was determined using a one-stage clotting assay kit (Dade Behring,
Fort Lauderdale, FL, USA). The plasma levels of prothrombin frag-
ment 1+2 (F1+2) and PAI-1 antigen were determined using
enzyme-immunosorbent assay kits for F1+2 (Behringwerke AG,
Marburg, Germany), and PAI-1 (Biopool, Umea, Sweden).20

The inter-assay coefficients of variation were 2.5% for the fibrinogen
assay, 3.6% for the F1+2 assay, and 5.9% for the PAI-1 assay.

Because these haemostatic parameters showed a skewed distri-
bution (see Supplementary material online, Figure S1), they were log-
arithmically transformed before analysis. The methods used to
measure other laboratory parameters are described in Supplemen-
tary material online.

Follow-up and events

The patients’ medical records were intermittently reviewed after
ABPM to check on their antihypertensive drug therapy and to
assess the occurrence of clinical stroke events. The follow-up was
performed during a 20-month period from 1996 to 1998; the
mean follow-up period was 41 months, with a range of 1–68
months.3,16 Of these, 292 patients (57%) were taking antihyperten-
sive medication at the time of the final follow-up. When patients
failed to come to the clinic, we interviewed them by telephone:
none of the cases interviewed by telephone were diagnosed as
having had a stroke event. All stroke events were diagnosed by
the physician who was caring for the patient at the time of the event,
and independent neurologists reviewed the cases and confi-
irmed the diagnosis of stroke events. Stroke was diagnosed on
the basis of a sudden onset of a neurological deficit persisting for
≥24 h in the absence of any other disease that could explain the
symptom. Stroke events were categorized as ischaemic stroke (cere-
bral infarction and cerebral embolism) or haemorrhagic stroke; the
patients whose diagnoses were defined by clinical symptoms but not
with brain computed tomography or MRI were considered to have
had an undefined type of stroke. We excluded transient ischaemic
attacks, i.e. those in which the neurological deficit was cleared com-
pletely within 24 h from the onset of symptoms.

Statistical analysis

All statistical analyses were performed with SPSS version 18.0J soft-
ware (SPSS, Chicago, IL, USA). Variables with normal distribution are
expressed as means ± SD, whereas variables with a skewed distri-
bution were logarithmically transformed before analysis and expressed
as geometric means (SD range). Clinical parameters in patients with or
without stroke events were compared using the unpaired t-test, and
categorical parameters were compared with the χ² test. Analysis of
covariance (ANCOVA) was performed to examine the differences in
haemostatic risk factors in patients with or without stroke events.
The Bonferroni test was used for pair-wise comparisons. The hazard
ratio (HR) and the 95% confidence interval (CI) of clinical stroke
events in each patient with quartiles [highest quartile (Q4) vs. lower quartiles (Q1–3)] of haemostatic risk factors and the morning BP surge were calculated using Cox regression analyses with adjustments for significant covariates. Finally, we divided the patients into three sets, each containing four groups: one set according to the circulatory levels with or without the highest quartile of F1+2 and PAI-1; another set, according to the circulatory levels with or without the highest quartile of F1+2 and the morning BP surge; and a third set, with or without the highest quartile of PAI-1 and morning BP surge. The cumulative incidences of clinical stroke events among these sets of four groups were plotted as Kaplan–Meier curves, and the differences were assessed by the log-rank test. Statistical significance was defined as \( P < 0.05 \).

Results
Clinical characteristics
The mean age ± SD of the subjects \((n = 514)\) was 72.3 ± 8.7 years, and 191 of the subjects (37%) were men. There were 257 patients who had SCI on brain MRI at the study recruitment. The clinical characteristics according to the SCI status are shown in Supplementary material online, Table S1.

Haemostatic risk factors and silent cerebral infarction
There were significant associations between circulatory fibrinogen and noradrenalin levels \((r = 0.115, P = 0.009)\), and between circulatory PAI-1 and adrenalin levels \((r = 0.114, P = 0.01)\); however, no association was found between haemostatic risk factors and the extent of the morning BP surge (data not shown). Patients with SCI had significantly higher levels of fibrinogen, F1+2, and PAI-1 than those without SCI, even after adjustment for confounding factors (see Supplementary material online, Table S2). Using multiple logistic regression analysis, the OR for the presence of SCI was estimated; the highest quartile of fibrinogen \((\geq 365 \text{ g/L}, n = 128)\) or PAI-1 (PAI-1 \(\geq 58 \text{ ng/mL}, n = 128\)) was significantly associated with the presence of SCI even after adjustment for confounding factors (see Supplementary material online, Table S3).

Haemostatic risk factors, morning blood pressure surge, and stroke events
During an average duration of 41 months (range: 1–68 months, 1751 person-years), 43 stroke events occurred (ischaemic, 30; haemorrhagic, 5; undefined, 8). Table 1 shows the differences in patient characteristics of the study population

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Stroke event (−) (n = 471)</th>
<th>Stroke event (+) (n = 43)</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>71.8 ± 8.7</td>
<td>76.9 ± 6.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male (%)</td>
<td>36</td>
<td>49</td>
<td>0.102</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.2 ± 3.5</td>
<td>23.4 ± 4.1</td>
<td>0.143</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>21</td>
<td>35</td>
<td>0.053</td>
</tr>
<tr>
<td>Type 2 diabetes (%)</td>
<td>14</td>
<td>19</td>
<td>0.372</td>
</tr>
<tr>
<td>Hyperlipidaemia (%)</td>
<td>22</td>
<td>19</td>
<td>0.702</td>
</tr>
<tr>
<td>Antihypertensive drugs (%)</td>
<td>58</td>
<td>42</td>
<td>0.053</td>
</tr>
<tr>
<td>Calcium-channel blockers (%)</td>
<td>40</td>
<td>23</td>
<td>0.033</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme-inhibitors (%)</td>
<td>21</td>
<td>21</td>
<td>1.000</td>
</tr>
<tr>
<td>Diuretics (%)</td>
<td>4</td>
<td>5</td>
<td>0.692</td>
</tr>
<tr>
<td>Antiplatelet drugs (%)</td>
<td>34</td>
<td>51</td>
<td>0.030</td>
</tr>
<tr>
<td>Statin (%)</td>
<td>17</td>
<td>19</td>
<td>0.831</td>
</tr>
<tr>
<td>BP measurement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Office SBP (mmHg)</td>
<td>162.2 ± 17.9</td>
<td>167.1 ± 19.2</td>
<td>0.003</td>
</tr>
<tr>
<td>24 h SBP (mmHg)</td>
<td>135.8 ± 16.8</td>
<td>141.1 ± 16.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Morning SBP surge (mmHg)</td>
<td>32.7 ± 17.2</td>
<td>40.8 ± 19.0</td>
<td>0.003</td>
</tr>
<tr>
<td>Laboratory data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose* (mg/dL)</td>
<td>92.3 (74.0–115.1)</td>
<td>93.8 (74.7–117.9)</td>
<td>0.637</td>
</tr>
<tr>
<td>Total cholesterol* (mg/dL)</td>
<td>199.4 (167.0–238.1)</td>
<td>190.1 (162.1–222.8)</td>
<td>0.087</td>
</tr>
<tr>
<td>Triglycerides* (mg/dL)</td>
<td>129.3 (79.1–211.2)</td>
<td>114.9 (73.9–178.5)</td>
<td>0.128</td>
</tr>
<tr>
<td>High-density lipoprotein* (mg/dL)</td>
<td>45.2 (34.8–58.8)</td>
<td>47.5 (36.5–61.7)</td>
<td>0.252</td>
</tr>
<tr>
<td>Adrenalin* (pg/mL)</td>
<td>33.5 (17.7–63.6)</td>
<td>41.4 (22.9–74.7)</td>
<td>0.038</td>
</tr>
<tr>
<td>Noradrenalin* (pg/mL)</td>
<td>337.4 (194.4–585.8)</td>
<td>511.5 (330.9–790.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>56.7 ± 13.2</td>
<td>55.2 ± 13.4</td>
<td>0.198</td>
</tr>
<tr>
<td>Prevalence of SCI at baseline (%)</td>
<td>47</td>
<td>86</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as the means ± SD. \(P\)-values were obtained by an unpaired t-test or \(\chi^2\) test. Variables with skewed distributions (asterisks*) were logarithmically transformed before analysis and expressed as geometric means (SD range).

SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; SCI, silent cerebral infarction.
characteristics and BP or laboratory data between patients who had clinical stroke events and those who did not. The prevalence of diabetes and that of hyperlipidaemia were the same between the two groups.

Baseline haemostatic risk factors among the patients with or without clinical stroke events are shown in Table 2. Both F1+2 and PAI-1 levels were significantly higher in stroke patients than in those without stroke events. In Cox regression analysis (Table 3), patients with the highest quartile of F1+2 or PAI-1 levels had a significantly higher incidence of clinical stroke events than those with lower quartiles of each haemostatic parameter, even after adjustment for confounding factors (Model 1). These associations remained significant even after adjusting for the morning BP surge (Table 3, Model 2), the presence of SCI (Model 3), or both (Model 4), and after adjusting for the use of an antihypertensive medication (or the use of a calcium-channel blocker) at the time of final follow-up (Model 5). The results in Table 3 were also adjusted for antiplatelet drugs and statin use, and these adjustments did not change the results. We divided the patients into four groups according to the circulatory levels of F1+2 and PAI-1, and the morning BP surge level. Kaplan–Meier cumulative incidences for clinical stroke events among each set of four groups are shown in Figure 1. We also analysed the HR and 95% CI of the patients for each of the four groups calculated by Cox regression analysis with adjustment for confounding factors (see Supplementary material online, Table S4); the patients who had the highest quartile of both F1+2 and PAI-1 levels had a higher risk of clinical stroke events than those with the lower quartiles of both F1+2 and PAI-1 levels (HR: 8.2; 95% CI: 3.7–18.2; P < 0.001), than those with the highest quartile of PAI-1 and the lower quartiles of F1+2 levels (HR: 4.5; 95% CI: 1.9–10.8; P = 0.001), and than those with the highest quartile of F1+2 and lower quartiles of PAI-1 levels (HR: 5.6; 95% CI: 2.2–14.3; P < 0.001) even after adjustment for significant covariates. Moreover, among the patients with the highest quartile of the morning BP surge, the multivariable HR (95% CI) for the highest vs. lower quartiles of PAI-1 was 3.4 (1.3–9.1), and that for F1+2 was 3.3 (1.3–8.7) (both P < 0.05).

**Time of stroke onset**

We identified the time of onset in 35 of the 43 events. Ten (63%) of 16 stroke events in the patients with the highest quartile of the morning BP surge occurred in the morning (6 a.m. to noon), whereas 7 (37%) of the 19 events in the lower quartiles of the morning BP surge occurred in this period (P = 0.181 by χ² test). In contrast, 9 (45%) of 20 stroke events in the patients with the highest quartile of PAI-1 levels occurred in the morning, whereas 8 (53%) of the 15 events in the patients with the lower quartiles of PAI-1 level occurred in this period (P = 0.738 by χ² test); a similar pattern was observed when the analysis was performed by the F1+2 level (data not shown). We also examined whether there was a linear tendency in the incidence of stroke events subdivided by the quartiles of each parameter; however, no linear tendency was found for any of the parameters (data not shown).

### Table 2 Baseline haemostatic parameters of patients with or without clinical stroke event

<table>
<thead>
<tr>
<th>Variables</th>
<th>Stroke (−) (n = 471)</th>
<th>Stroke (+) (n = 43)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>266.5 (261.2–271.9)</td>
<td>285.7 (266.6–306.0)</td>
<td>0.059</td>
</tr>
<tr>
<td>Prothrombin fragment 1 + 2 (nmol/L)</td>
<td>1.46 (1.40–1.52)</td>
<td>1.78 (1.56–2.04)</td>
<td>0.004</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor-1 (ng/mL)</td>
<td>38.1 (36.1–40.3)</td>
<td>51.7 (42.7–52.5)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Variables were logarithmically transformed before analysis and expressed as the geometric mean (95% CI) with adjustment for age, sex, current smoking, a history of diabetes or hyperlipidaemia, antihypertensive medication at the time of final follow-up, and 24 h SBP level by ANCOVA.

### Table 3 Cox regression analysis for stroke events

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1 HR (95% CI)</th>
<th>Model 2 HR (95% CI)</th>
<th>Model 3 HR (95% CI)</th>
<th>Model 4 HR (95% CI)</th>
<th>Model 5 HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning BP surge (10 mmHg)</td>
<td>—</td>
<td>1.2 (1.1–1.5)</td>
<td>—</td>
<td>1.2 (1.1–1.4)</td>
<td>1.2 (1.1–1.4)</td>
</tr>
<tr>
<td>Silent cerebral infarction (0 = no, 1 = yes)</td>
<td>—</td>
<td>—</td>
<td>3.1 (1.3–7.7)</td>
<td>2.9 (1.2–7.1)</td>
<td>2.9 (1.2–7.2)</td>
</tr>
<tr>
<td>Haemostatic parameters</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>1.4 (0.8–2.7)</td>
<td>1.4 (0.7–2.7)</td>
<td>1.3 (0.7–2.5)</td>
<td>1.3 (0.7–2.5)</td>
<td>1.3 (0.7–2.5)</td>
</tr>
<tr>
<td>Prothrombin fragment 1 + 2</td>
<td>2.7 (1.4–5.1)</td>
<td>2.8 (1.3–5.2)</td>
<td>2.7 (1.4–5.1)</td>
<td>2.6 (1.4–5.0)</td>
<td>2.6 (1.4–5.0)</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor-1</td>
<td>3.3 (1.8–6.0)</td>
<td>3.2 (1.7–5.8)</td>
<td>2.7 (1.5–5.1)</td>
<td>2.7 (1.4–4.9)</td>
<td>2.5 (1.3–4.6)</td>
</tr>
</tbody>
</table>

Hazard ratios and 95% CI for stroke events are shown after adjustment for age, sex, body mass index, current smoking, a history of diabetes or hyperlipidaemia, serum noradrenalin level, and 24 h SBP level. The analysis of each haemostatic parameter was based on the following code: 0 = the lower three quartiles, 1 = the highest quartile. Additional adjustment for antihypertensive medication at the time of final follow-up in Model 4 was performed and the results are shown in Model 5.

P < 0.05.

P < 0.01.

P < 0.001.
shown). Of the 43 stroke events, 30 were ischaemic, 5 were haemorrhagic, and 8 were of unknown type. There was no significant difference in the stroke subtype between the highest quartile and the lower quartiles of the morning BP surge, PAI-1, or F1+2 levels.

Discussion

In Japan, the mortality and morbidity of stroke are higher than those of CAD, whereas the opposite is true in western countries. Thus, the contributors to stroke events must be elucidated to minimize the incidence of stroke in the Japanese people.

In contrast to CAD, only limited data on stroke events are available for examining the association between plasma markers of coagulation and/or fibrinolysis, especially in Japan. As in previous reports from western countries, our data showed that both F1+2, a marker of thrombin generation, and PAI-1, a principal inhibitor of fibrinolysis, were significantly associated with stroke events independently of 24 h BP levels and the presence of SCI at baseline. In particular, the stroke risk was substantially higher in patients with the highest quartile of both PAI-1 (≥58 ng/mL) and F1+2 (≥1.78 nmol/L) compared with those with the highest quartile of PAI-1 or F1+2 alone. This may indicate that the haemostatic imbalance resulting from impaired fibrinolytic activity, in combination with the enhanced procoagulant mechanism, increases the incidence of stroke events.

The circulating PAI-1 level is determined by a number of factors, including age, body mass index, smoking, insulin resistance syndrome, and some neurohumoral risk factors (SNA and RAAS). Our data show, however, that the PAI-1 level remained significantly associated with stroke events even after accounting for some confounding factors. As in our previous report, we also observed that the PAI-1 level was associated with the presence of SCI at baseline in this study. This means that circulating PAI-1 may not only contribute to the triggering of clinical stroke events, but may also accelerate microthrombosis formation in the small arteries even in the silent stage of hypertensive cerebrovascular disease. Intriguingly, some human autopsy studies have revealed the local expression of certain haemostatic risk factors.

Figure 1 Kaplan–Meier cumulative incidence of stroke. Kaplan–Meier cumulative incidence of clinical stroke events in patients with or without the highest quartile of plasminogen activator inhibitor-1 and/or prothrombin fragment 1+2 (A), plasminogen activator inhibitor-1 and/or morning blood pressure surge (B), and prothrombin fragment 1+2 and/or morning blood pressure surge (C).
such as tissue plasminogen activator and PAI-1, in the cerebral microvascular endothelium.\textsuperscript{20,21} The disturbance of such local haemostasis in addition to the high level of circulating PAI-1 may contribute to microthrombosis formation in humans.

In previous studies on hypertensive patients, a prothrombotic or hypercoagulable condition was observed when the BP level was poorly controlled or when concomitant target-organ damage (TOD), such as cardiac hypertrophy, arterial stiffness, or renal dys-

function, was present.\textsuperscript{22} Since these previous studies were cross-

sectional, they could not address the question of whether the association between the hypercoagulable state and TOD was one of cause or effect—i.e. whether a hypercoagulable state is a consequence or a cause of TOD. However, our present prospective data raise the possibility that a hypercoagulable state is not merely a marker or consequence of TOD but may contribute to the pathogenesis of cardiovascular events in hypertensive patients.

To the best of our knowledge, our study is the first to demonstrate that the plasma F1+2 level is a useful marker for predicting stroke events independently of baseline SCI status in Japanese hypertensive patients. This suggests that thrombin has an active pathological role in the occurrence of stroke in older hypertensive patients. In contrast, the plasma fibrinogen level could not predict stroke events in our study; this finding was inconsistent with western reports,\textsuperscript{7} but was consistent with recent Japanese reports conducted from a general population. Those reports showed that the plasma fibrinogen level was associated with the presence of SCI,\textsuperscript{23} but not with stroke events.\textsuperscript{9} This discrepancy between western and Japanese reports might be due to a difference in the subtypes of stroke among them; i.e. lacunar infarction is the most common subtype in Japan, while atherothrombotic infarction is dominant in Western countries.\textsuperscript{24} The pathogenesis of these two subtypes differs, and the contribution of the fibrinogen level to cerebrovascular disease seems to be lower in lacunar stroke than in non-lacunar stroke.\textsuperscript{25} In the present study, we could not represent the ischaemic stroke subtypes separately, and thus we were unable to determine the effect of fibrinogen on stroke incidence according to the aetiological subtype of ischaemic stroke. Studies with more accurate subtyping of stroke events during follow-up are required.

In the present study, both an excessive morning BP surge and haemostatic risk factors were independently and additively associated with an increased risk of stroke in older hypertensive patients. This means that their combined presence represented a greater stroke risk than that conferred by either the morning BP surge or haemostatic risk factors alone, and this additive effect may indicate that the morning BP surge and haemostatic risk factors participate in different pathophysiological processes in the development of cerebrovascular disease. Both the morning BP surge and certain haemostatic risk factors are affected by numerous physiological and pathophysiological factors, including neurohumoral factors such as SNA and RAAS, which are thought to be important determinants of stroke onset;\textsuperscript{213–15} however, in the present study, the risk of the morning BP surge and haemostatic risk factors remained unchanged even after adjustment for serum adrenaline and noradrenaline levels (Supplementary material online, Table S4), which suggests that the cerebrovascular risk conferred by morning BP surge, haemostatic risk factors, or both, was independent of SNA.

Plasma F1+2 and PAI-1 levels have been shown to be elevated in the morning.\textsuperscript{2,10,11} Therefore, we hypothesize that a morning hypercoagulable or hypofibrinolytic condition, together with the morning BP surge, may contribute considerably to the morning increase in cardiovascular events.\textsuperscript{2} In contrast to our previous reports,\textsuperscript{7} in our present study the prevalence of the morning onset (6 a.m. to noon) of stroke events was not statistically differ-

ent between patients with the highest quartile of the morning BP surge and those with lower quartiles, although stroke events showed a greater tendency to cluster in the morning in patients with an excessive morning BP surge than in those without it (63 vs. 37%). This discrepancy was attributed to the fact that the highest quartile of the morning BP surge was lower in the present study than in our previous reports (>44 vs. ≥55 mmHg). In contrast, this phenomenon was not observed in the patients with the highest quartile of PAI-1 or F1+2 levels. This may have been due to the fact that we measured haemostatic risk factors at only one point. Therefore, we could not assess whether the high levels of the morning PAI-1 or F1+2 were derived from the morning increase over night-time levels (or over other periods) or a persistent elevation throughout the day, even though the circadian variation in these haemostatic parameters was characterized by an increase in the morning.\textsuperscript{2,10,11} This point should be investigated in the future.

The strength of this study was its simultaneous prospective analysis of data on the morning BP surge (i.e. haemodynamic factors), molecular markers of haemostatic activation, and MRI-detected SCI within the same hypertensive patients; however, there were also several limitations. First, the number of stroke events was relatively small, and thus we could not separate stroke events into those of an ischaemic and those of a haemor-

hagic origin. This point should be examined using a larger database in the future. Second, single measurements of BP and haemostatic risk factors may lead to regression dilution bias and thus may underestimate the strength of associations.\textsuperscript{26} Third, new-onset atrial fibrillation during the follow-up periods was not assessed in this study, although it could have substantially contributed to the stroke incidence in hypertensive patients. Lastly, the lesser use of calcium-channel blockers and the greater use of antplatelet drugs in the patients with clinical stroke events compared with the patients without stroke events were potentially confounding. Although our results were statistically adjusted by these factors, residual confounding due to unknown or incompletely measured characteristics could not be excluded in the present study.

In conclusion, this study has clearly shown that high levels of plasma PAI-1 and F1+2, as well as an excessive morning BP surge, are independently and additively associated with an increased risk of stroke in older hypertensive patients. These parameters might be useful in the diagnostic work-up of older hypertensive patients to identify those who have likely developed or will develop cerebrovascular diseases. Further studies are needed to confirm these results. In particular, interventional studies will be able to establish the precise values of these parameters.

**Supplementary material**

Supplementary material is available at European Heart Journal online.
Funding

This study was supported by a Grant-in-Aid from the Foundation for the Development of the Community (K.K.), a Research Grant for Cardiovascular Medicine (14–6) from the Ministry of Health, Labour, and Welfare (K.K.), and a Research Grant (C-2) from the Ministry of Education, Culture, Sports, Science, and Technology (K.K.), Japan.

Conflict of interest: none declared.

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