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Putative Role of Asymmetric Dimethylarginine in Microvascular Disease of Kidney and Heart in Hypertensive Patients

Hideki Fujii[1,2], Shin Takiuchi[2], Yuhei Kawano[2] and Masafumi Fukagawa[1]

BACKGROUND

Despite the frequent simultaneous presentation of cardiac and renal dysfunction, the relationship between these pathophysiological processes remains unclear. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide (NO) synthase, which has been linked to endothelial dysfunction and atherosclerosis. This study elucidates the relationship between ADMA and intrarenal and coronary microvascular diseases.

METHODS

In this study, we included 66 consecutive hypertensive patients with normal renal function or mild renal insufficiency (creatinine ≤ 1.2 mg/dl). On the basis of their estimated glomerular filtration rate (eGFR), the patients were divided into two groups (normal group, eGFR ≥90 ml/min; renal insufficiency group, eGFR <90 ml/min). Coronary flow velocity reserve (CFVR) was measured using adenosine-triphosphate stress transthoracic Doppler echocardiography. In addition, a plasma ADMA assay, echocardiography, carotid ultrasound, and brachial-ankle pulse wave velocity measurement were performed.

RESULTS

The plasma ADMA level was the highest in patients with both renal insufficiency and reduced CFVR. ADMA was significantly associated with eGFR ($r = -0.342$, $P = 0.006$) and CFVR ($r = -0.459$, $P < 0.001$), and eGFR and CFVR were significantly associated with each other ($r = 0.337$, $P = 0.006$). Multiple regression analysis revealed that ADMA was an independent clinical parameter associated with both eGFR and CFVR.

CONCLUSIONS

Plasma ADMA is suggested to be an incipient biochemical marker of microvascular disease in both kidney and heart in hypertensive patients. ADMA might play an important role in the pathogenesis of organ damage in the kidney and heart in essential hypertension.

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Patients with chronic kidney disease (CKD) have a higher risk of developing cardiovascular disease (CVD) than the general population. It has been reported that not only end-stage renal disease but even mild renal insufficiency is associated with increased risk of CVD.[1,2] Although CKD may be considered as an independent risk factor for CVD, the association between the renal and cardiovascular systems remains unclear.

An impairment of coronary flow velocity reserve (CFVR) as a result of microvascular abnormalities has been reported in various models of preclinical disease in the absence of coronary atherosclerosis.[3–5] We have reported earlier that CFVR decreases in hypertensive patients who experience chest pain but have no coronary artery disease.[6] CFVR is thought to decrease because of impaired coronary microcirculation. Similarly, renal function depends on the integrity of the microvascular beds. It follows, therefore, that glomerular filtration rate (GFR) may be associated with renal microcirculation. It has been reported that mild renal insufficiency is associated with reduced coronary flow in patients with no coronary artery disease.[7] This relationship may indicate parallel alternations in coronary and renal microcirculation.

Many studies have demonstrated that nitric oxide (NO) plays an important role in the progression of atherosclerosis.[8] NO is synthesized by endothelial, neuronal, and macrophage isoforms of the enzyme NO synthase. Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NO synthase, and the serum ADMA level has been suggested to be a surrogate marker of endothelial dysfunction and/or angiosclerosis. Recently, we revealed the relationship between ADMA and coronary and peripheral endothelial dysfunction.[9] Several studies have demonstrated that serum ADMA levels increase in CKD patients, and are strongly related to the severity of the atherosclerotic diseases.[10–12] In view of these findings, ADMA is thought to be a novel biochemical marker of endothelial dysfunction and/or vascular lesions.

[1]Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe, Japan; [2]Division of Hypertension and Nephrology, National Cardiovascular Center, Suita, Japan. Correspondence: Masafumi Fukagawa (fukagawa@med.kobe-u.ac.jp)

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This study was initiated to examine the relationship between ADMA, GFR, and CFVR in patients with normal renal function or mild renal insufficiency, and with normal coronary artery function or mild coronary artery disease. We hypothesized that ADMA may be involved in the underlying mechanism connecting the two pathologic conditions, and may be an incipient biochemical marker of microvascular disease of kidney and heart in hypertensive patients.

METHODS

Study population. The study population consisted of 66 consecutive patients with essential hypertension, without significant coronary artery stenosis. All the patients had been hospitalized in our institution between 2003 and 2004. Eleven of the patients had participated in our previous study.[9] All the hypertensive patients had a well-established history of elevated casual blood pressure (BP) >140/90 mm Hg, on the basis of at least three sets of readings taken at 1-month intervals. The exclusion criteria for this study included the presence of macrohematuria, proteinuria, renal artery stenosis, coronary artery disease, valvular heart disease, cardiomyopathy, peripheral artery disease, old cerebral infarction, diabetes mellitus, secondary hypertension, and insufficient echo imaging of the coronary arteries. Moreover, patients with arrhythmias, including atrioventricular blocks or atrial fibrillation, and bronchial asthma were excluded because the administration of adenosine triphosphate could have worsened their symptoms. Coronary artery disease was ruled out by negative findings on exercise stress electrocardiography, scintigraphy, or coronary angiography. All the patients with positive findings on these examinations or CFVR <2.0 underwent coronary angiography. The presence of peripheral artery disease was determined on the basis of subjective and objective complaints and findings arising from leg ischemia and/or ankle-brachial index <0.9. Renal artery stenosis was screened for, using Duplex ultrasonography. Diabetes mellitus was defined as a patient's use of oral hypoglycemic agents or insulin and/or having a fasting glucose level >126 mg/dl or a random non-fasting blood glucose level >200 mg/dl.

In patients taking antihypertensive drugs, medication was withdrawn ~1 week before the examination so as to exclude direct effects on vasodilation. Individuals with a smoking habit abstained from smoking for 1 week before the examination. All the patients abstained from caffeine the day before the examination. BP was measured by trained personnel using a calibrated mercury sphygmomanometer with appropriate cuffs (two sizes), with the subject in a supine position, and ensuring standardized conditions. Three readings were taken and the average of the last two readings was used for the analyses. The experimental protocols were approved by the appropriate institutional review committee and informed consent was obtained from all the subjects. This study was performed prospectively and all the examinations were conducted by several investigators who were blinded to the patients' characteristics.

We divided the patients into two groups on the basis of their estimated GFR (eGFR). Patients with eGFR ≥90 ml/min were placed in group A (n = 33; normal or CKD stage 1), while those with eGFR ≤90 ml/min were placed in group B (n = 33; CKD stages 2 or 3).

Determination of GFR. GFR was estimated using the Cockroft and Gault equation, eGFR = (140−age) × body weight/(72 × serum creatinine). A correction factor of 0.85 was used for female subjects.

Measurement of CFVR. CFVR was measured using transthoracic Doppler echocardiography, in the manner described earlier.[9] Briefly, transthoracic Doppler echocardiography examinations were conducted using a Siemens Sequoia digital ultrasound system at a frequency of 7.0 MHz (Siemens USA, Mountain View, CA). The ultrasound beam was transmitted toward the heart to visualize coronary blood flow in the distal portion of the left anterior descending coronary artery by color Doppler flow mapping. First, the left ventricle was imaged in cross-section along the longitudinal axis, and then the ultrasound beam was inclined laterally. Next, coronary blood flow in the distal left anterior descending was examined under the guidance of color Doppler flow mapping. After positioning a sample volume on the color signal in the distal left anterior descending, Doppler spectral tracings of flow velocity were recorded using fast Fourier transformation analysis. All the results were recorded on 0.5-inch S-VHS videotapes for off-line analysis.

We first recorded baseline spectral Doppler signals in the distal left anterior descending. Adenosine triphosphate was administered (140 $\mu g\,kg^{-1}\,min^{-1}$ IV) for 3 min to record spectral Doppler signals during hyperemic conditions. All the patients underwent continuous heart rate and BP monitoring throughout the study period.

Analysis of coronary flow velocity was conducted off-line by tracing the contour of the spectral Doppler signal using an ultrasound system computer. Mean diastolic velocity was measured at baseline and peak hyperemic conditions. The measurements were averaged over three cardiac cycles. CFVR was defined as the ratio of hyperemic to basal mean diastolic velocity. We adopted a CFVR of <2.0 as the cutoff value for the presence of significant coronary microvascular disease, as in the previous studies.[13]

Echocardiographic study. Two-dimensional guided M-mode echocardiography was conducted to measure left ventricular wall mass. Left ventricular diastolic and systolic diameters (LVDd/LVDs), and the diastolic thickness of the left ventricular posterior wall (LVPWT) and interventricular septum (IVST), were assessed in M-mode images in the parasternal longitudinal-axis view. The M-mode analysis was conducted in accordance with the guidelines of the American Society of Echocardiography. The left ventricular mass index (LVMI) (g/m^2) was calculated using the formula:[14]

$$\mathrm{LVMI}(g/m^2) = \frac{1.04 \times [(\mathrm{IVST} + \mathrm{LVPWT} + \mathrm{LVDd})^3 - \mathrm{LVDd}^3] - 13.6}{\text{body surface area}}$$

Carotid ultrasonographic study. The subjects were investigated while in the supine position with the head slightly turned from

the sonographer. The carotid arteries were carefully examined for wall changes, from different longitudinal (anterior oblique, lateral, and posterior oblique) and transverse views. The common carotid arteries were examined in all the subjects. A region about 15 mm proximal to the carotid bifurcation was identified, and the intima-media thickness (IMT) of the far wall was evaluated as the distance between the luminal-intimal interface and the medial-adventitial interface. One transversal and two longitudinal measurements of IMT were obtained from 10 contiguous sites at 1-mm intervals, and the average of the 10 measurements was used for the analysis. The IMT was measured at a site free of any discrete plaques.

Pulse wave velocity measurement. Brachial-ankle pulse wave velocity was measured using a volume-plethysmographic apparatus (Form/ABI; Colin, Komaki, Aichi, Japan). The subjects were examined while resting in the supine position. Electrocardiographic electrodes were placed on both wrists, and cuffs were wrapped on the bilateral brachia and ankles. Pulse volume waveforms at the brachium and ankle were recorded using a semiconductor pressure sensor after a rest period of at least 5 min.

Measurement of plasma ADMA levels and other laboratory determinations. On the day the CFVR measurement was to be recorded, venous blood was collected from the patients after a 20-min period of supine rest in the morning, following overnight fasting. Blood was drawn into chilled citrate tubes on ice. Plasma was separated by centrifugation at 2,500 *g* for 10 min at 4 °C and stored at −20 °C until analysis. Plasma ADMA levels were determined at Fujimoto Biomedical Laboratories (Matsubara, Osaka, Japan) using a novel high-performance liquid chromatography method. This method used the Hitachi L-7480 system (Hitachi, Tokyo, Japan) equipped with a fluorescence detector for excitation at 348 nm and emission at 450 nm with an ODS column using ortho phthaldialdehyde for fluorescence determination. Other laboratory tests were conducted using standardized clinical laboratory methods.

Statistical analysis. We used the computer software application StatView 5.0 (SAS Institute, Cary, NC) for all statistical analyses. Values are presented as mean ± s.d. The significance of the differences between two groups was analyzed by the Student's *t*-test for continuous variables and by the χ^2-test for categorical variables. The differences among three groups were examined by analysis of variance. Relationships between variables were assessed using univariate linear regression analysis. In order to determine the independent biochemical markers for GFR and CFVR, we performed a multiple regression analysis. A *P* value of <0.05 was considered statistically significant.

RESULTS

Patients' characteristics

The main characteristics of the subjects are shown in **Table 1**. The patients in group B (CKD stages 2 or 3) were older than those in group A (normal or CKD stage 1). As for the other clinical parameters measured, in group B, eGFR was significantly lower, and IMT, brachial-ankle pulse wave velocity and A/E ratio were significantly higher than in group A. Before withdrawal of antihypertensive drugs, there were no significant differences in BP between the two groups (group A, systolic/diastolic BP: 127 ± 16/76 ± 13 mm Hg; group B, systolic/diastolic BP: 131 ± 28/79 ± 28 mm Hg).

Plasma ADMA concentrations and microvascular disease

The plasma ADMA level was significantly higher in group B than in group A. In order to further evaluate the relationship

Table 1 | Clinical characteristics of the patients

Clinical characteristics	All patients (*n* = 66)	Group A (*n* = 33)	Group B (*n* = 33)	*P*
Age (years)	62 ± 10	57 ± 10	66 ± 6	<0.001
Male (%)	31 (47.0)	18 (54.5)	13 (39.4)	0.224
Body weight (kg)	62.0 ± 11.7	68.2 ± 9.8	58.0 ± 9.6	<0.001
BMI (kg/m^2)	24.7 ± 3.4	25.8 ± 3.3	23.5 ± 3.2	0.005
Smoking (%)	57 (86.4)	28 (84.8)	29 (87.9)	0.919
Hyperlipidemia (%)	23 (34.8)	14 (42.4)	9 (27.3)	0.202
SBP (mm Hg)	151 ± 23	144 ± 16	160 ± 26	0.003
DBP (mm Hg)	86 ± 14	85 ± 14	87 ± 13	0.534
Cr (mg/dl)	0.77 ± 0.19	0.73 ± 0.17	0.82 ± 0.19	0.043
BUN (mg/dl)	16.1 ± 2.0	15.8 ± 4.0	16.5 ± 4.1	0.487
Total cholesterol (mg/dl)	195.6 ± 29.8	196.7 ± 32.0	194.6 ± 27.9	0.778
TG (mg/dl)	116.5 ± 48.7	130.0 ± 50.8	103.0 ± 43.2	0.024
HDL-C (mg/dl)	50.3 ± 16.4	47.6 ± 14.7	52.9 ± 17.7	0.196
LDL-C (mg/dl)	122.1 ± 31.7	123.0 ± 32.3	121.1 ± 31.4	0.805
Glucose (mg/dl)	96.3 ± 13.9	93.5 ± 13.0	99.1 ± 14.4	0.103
HbA1c (%)	5.5 ± 0.8	5.4 ± 0.7	5.7 ± 1.0	0.120
PRA (ng/ml·hr)	1.2 ± 1.3	0.9 ± 0.8	1.5 ± 1.6	0.100
PAC (ng/dl)	15.2 ± 7.4	14.6 ± 8.1	15.7 ± 6.9	0.563
ET-1 (pg/ml)	3.44 ± 1.32	3.15 ± 1.17	3.73 ± 1.41	0.073
ADMA (nmol/ml)	0.51 ± 0.07	0.49 ± 0.06	0.53 ± 0.08	0.043
IRI (mU/l)	6.2 ± 4.0	5.4 ± 3.8	6.9 ± 4.1	0.126
hsCRP (mg/dl)	0.135 ± 0.161	0.122 ± 0.169	0.149 ± 0.156	0.536
HOMA-R	1.58 ± 1.15	1.33 ± 1.09	1.85 ± 1.17	0.122
eGFR (ml/min)	94.9 ± 32.1	120.2 ± 23.9	69.5 ± 14.1	<0.001
IMT (mm)	1.46 ± 0.71	1.32 ± 0.64	1.75 ± 0.92	0.031
baPWV (cm/s)	1871.9 ± 452.2	1689.1 ± 390.7	2066.6 ± 436.6	<0.001
LVMI (g/m^2)	119.5 ± 26.2	119.4 ± 23.7	119.6 ± 28.4	0.972
A/E ratio	1.21 ± 0.29	1.12 ± 0.28	1.30 ± 0.28	0.012

Values are mean ± s.d. Group A, eGFR ≥90 ml/min; Group B, eGFR <90 ml/min.
ADMA, asymmetric dimethylarginine; baPWV, brachial-ankle pulse wave velocity; BMI, body mass index; BUN, blood urea nitrogen; Cr, creatinine; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ET-1, endothelin-1; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-R, homeostasis model assessment; hsCRP, high sensitivity C-reactive protein; IMT, intima-media thickness; IRI, immunoreactive insulin; LDL-C, low-density lipoprotein cholesterol; LVMI, left ventricular mass indexed for body surface area; PAC, plasma aldosterone concentration; PRA, plasma renin activity; SBP, systolic blood pressure; TG, triglyceride.

between plasma ADMA levels and coronary microvascular disease, we divided the study patients into two groups based on their CFVR value (normal CFVR ≥2.0; $n = 52$, abnormal CFVR <2.0; $n = 14$). Mean plasma ADMA levels were significantly higher in the patients with abnormal CFVR (group with CFVR ≥2.0; 0.49 ± 0.07 pg/ml, group with CFVR <2.0; 0.56 ± 0.06 pg/ml: **Figure 1a**). In addition, the patients in group B had significantly lower CFVR than those in group A (group A; 2.70 ± 0.63, group B; 2.30 ± 0.50: **Figure 1b**). Further, the patients were divided into the following three groups on the basis of the presence of microvascular disease: group 1, CFVR ≥2.0 and eGFR ≥90 ml/min; group 2, CFVR <2.0 or eGFR <90 ml/min; group 3, CFVR <2.0 and eGFR <90 ml/min. The plasma ADMA level tended to be higher in group 2 than in group 1, and it was significantly higher in group 3 than in groups 1 and 2 (**Figure 2**).

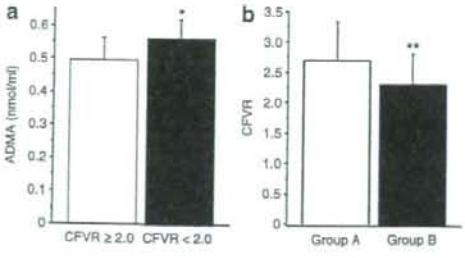

Figure 1 | Plasma asymmetric dimethylarginine (ADMA) concentration and microvascular disease. (**a**) Average values of plasma ADMA concentration in the group with coronary flow velocity reserve (CFVR) ≥2.0 and the group with CFVR <2.0. (**b**) Average values of CFVR in groups A and B. Values are shown as mean ± s.d. *$P = 0.003$; **$P < 0.001$.

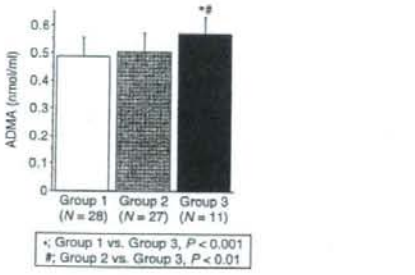

Figure 2 | Average values of plasma asymmetric dimethylarginine (ADMA) concentration in relation to the presence of microvascular disease. Group 1, CFVR ≥2.0 and eGFR ≥90 ml/min; Group 2, CFVR <2.0 or eGFR <90 ml/min; Group 3, CFVR <2.0 and eGFR <90 ml/min. Values are shown as mean ± s.d.

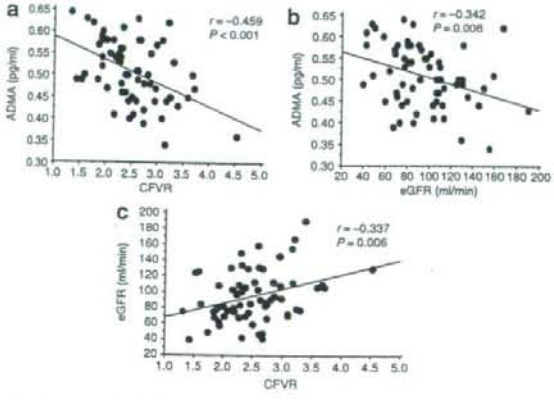

Figure 3 | Relationship between the three parameters: (**a**) Coronary flow velocity reserve (CFVR) and plasma asymmetric dimethylarginine (ADMA) concentration, (**b**) estimated glomerular filtration rate (GFR) and plasma ADMA concentration, and (**c**) CFVR and eGFR.

Correlation between ADMA, eGFR, and CFVR

Plasma ADMA levels were significantly associated with age ($r = 0.481$, $P < 0.001$), high sensitivity C-reactive protein ($r = 0.293$, $P = 0.030$), IMT ($r = 0.464$, $P < 0.001$), and A/E ratio ($r = -0.304$, $P = 0.020$). Further, ADMA had a significant correlation with both eGFR ($r = -0.342$, $P = 0.006$; **Figure 3a**) and CFVR ($r = -0.459$, $P < 0.001$; **Figure 3b**). We also found CFVR to be significantly correlated with eGFR ($r = 0.337$, $P = 0.006$; **Figure 3c**).

Univariate and multivariate predictors of eGFR and CFVR

The correlation coefficients between eGFR or CFVR and various clinical parameters were evaluated. The univariate analysis revealed that eGFR was significantly correlated with age ($r = -0.583$, $P < 0.001$), sex ($r = -0.240$, $P < 0.001$), body mass index ($r = -0.384$, $P = 0.002$), homeostasis model assessment ($r = -0.368$, $P = 0.011$), and ADMA ($r = -0.342$, $P = 0.006$), while CFVR was significantly correlated with age ($r = -0.434$, $P < 0.001$), sex ($r = -0.264$, $P = 0.032$), IMT ($r = -0.359$, $P = 0.003$), plasma aldosterone concentration ($r = 0.262$, $P = 0.037$), endothelin-1 ($r = -0.270$, $P = 0.028$), and ADMA ($r = -0.459$, $P < 0.001$). Multiple regression analysis was conducted between eGFR or CFVR and the clinical parameters listed in **Tables 2**

Table 2 | Analysis of the relationship between eGFR or CFVR and clinical parameters by multivariate linear regression; model 1

	eGFR		CFVR	
	β	P	β	P
Age	−0.514	0.002	−0.068	0.761
BMI	0.287	0.023	0.143	0.397
Sex	−0.175	0.200	−0.242	0.205
SBP	0.039	0.766	0.056	0.762
IMT	−0.006	0.966	0.076	0.699
A/E	0.055	0.413	0.084	0.658
HOMA-R	−0.125	0.307	−0.135	0.432
LDL-C	0.145	0.211	0.125	0.440
hsCRP	−0.086	0.486	−0.135	0.436
PAC	−0.139	0.306	0.228	0.235
ET-1	−0.116	0.336	−0.240	0.159
ADMA	−0.194	0.189	−0.407	0.048

ADMA, asymmetric dimethylarginine; BMI, body mass index; CFVR, coronary flow velocity reserve; eGFR, estimated glomerular filtration rate; ET-1, endothelin-1; HOMA-R, homeostasis model assessment; hsCRP, high sensitivity C-reactive protein; IMT, intima-media thickness; LDL-C, low density lipoprotein cholesterol; PAC, plasma aldosterone concentration; SBP, systolic blood pressure.

Table 3 | Analysis of the relationship between eGFR or CFVR and clinical parameters by multivariate linear regression; model 2

	eGFR		CFVR	
	β	P	β	P
BMI	0.360	0.016	0.153	0.351
Sex	−0.282	0.076	−0.255	0.162
SBP	−0.028	0.858	0.048	0.792
IMT	0.110	0.499	0.091	0.630
A/E	−0.014	0.931	0.075	0.682
HOMA-R	−0.202	0.163	−0.125	0.449
LDL-C	0.087	0.519	0.117	0.454
hsCRP	−0.154	0.289	−0.143	0.393
PAC	0.002	0.988	0.246	0.172
ET-1	−0.066	0.639	−0.234	0.159
ADMA	−0.403	0.015	−0.433	0.023

ADMA, asymmetric dimethylarginine; BMI, Body mass index; CFVR, coronary flow velocity reserve; eGFR, estimated glomerular filtration rate; ET-1, endothelin-1; HOMA-R, homeostasis model assessment; hsCRP, high sensitivity C-reactive protein; IMT, intima-media thickness; LDL-C, low density lipoprotein cholesterol; PAC, plasma aldosterone concentration; SBP, systolic blood pressure.

and 3. Body weight (or body mass index) and age greatly influence the results because they are used in the Cockroft and Gault equation for eGFR. The results of multiple regression analysis including these parameters showed that age and body mass index had a significant independent association with eGFR (**Table 2**). When age was not included in the model, only ADMA showed a statistically significant independent relationship with eGFR and CFVR (**Table 3**). When ADMA was added to a model with the other covariates, the absolute value of the magnitude of the regression coefficient for age was reduced by 23%.

DISCUSSION

Our study demonstrated that (i) the plasma ADMA level is already elevated at the early stage of kidney and heart disease in hypertensive patients; (ii) ADMA is significantly correlated with age, IMT, A/E ratio, and high sensitivity C-reactive protein; (iii) there is a significant association between ADMA, GFR, and CFVR; and (iv) ADMA is a statistically significant independent clinical parameter that is associated with both GFR and CFVR at the early stage of kidney and heart disease.

Recently, accumulating evidence has shown that not only end-stage renal disease but also minor renal dysfunction is a significant cardiovascular risk factor.[1] An American Heart Association statement published in 2003 recommended that patients with CKD should be considered as members of the highest risk group for subsequent CVD events.[15] It is thought that even mild renal dysfunction and/or the presence of albuminuria is correlated with increased cardiovascular mortality and morbidity.[16,17] Yet, despite growing recognition of the cardio-renal association, its detailed mechanisms are not well understood.

Impaired CFVR was thought to arise during advanced stages of disease, when left ventricular hypertrophy becomes obvious.[5] However, recent studies have suggested that CFVR decreases even at early disease stages in hypertensive patients without left ventricular hypertrophy.[4] Impaired blood flow in small intramural resistance vessels or in the coronary capillary system results in decreased coronary microcirculation. Opherk *et al.* suggested that the reduced CFVR in hypertensive patients may be attributed to abnormalities in small intramyocardial vessels that cannot be visualized by coronary angiography.[18] These abnormalities are currently considered to be a critical step in the development of angiosclerosis. Our recent study also showed similar deterioration in coronary and peripheral vascular territories.[9] These findings suggest that impaired CFVR could be a systemic early indicator of organ damage caused by microcirculation abnormalities. Our present findings also showed that CFVR decreased even in patients with mild renal insufficiency. That is, hypertensive patients may already have microvascular disease and/or atherosclerotic lesions before the appearance of overt renal and cardiac disease.

In the past few decades, many studies have revealed an important role for NO, a potent anti-atherosclerotic molecule, in the development of endothelial dysfunction.[14] Decreased local NO production leads to progressive damage because of impaired microcirculation in the kidneys, heart, and other systemic organs. The role of increased plasma ADMA levels and vascular injury has been studied in not only *in vivo* studies [19,20] but also in various clinical conditions such as diabetes, hypercholesterolemia, and CVD.[21–23] Suda *et al.*, in an experimental study, reported that long-term treatment with ADMA leads to coronary microvascular lesions.[20] In addition, Kielstein *et al.* demonstrated, in a study in humans, that systemic ADMA infusion decreases cardiac output and renal blood flow in a dose-related manner.[24] Therefore, elevation of plasma ADMA levels is thought to be the first step in the process of microvascular disease and/or atherosclerosis. Numerous studies have suggested that an increase in the concentration of plasma ADMA might account for endothelial dysfunction in patients with CKD.[11,25,26] Indeed, in patients with or without renal disease, elevated plasma ADMA levels have a strong relationship with the severity of the atherosclerotic disease. Further, high plasma ADMA levels in CKD patients have been associated with increases in the cardiovascular risk factors such as C-reactive protein, IMT, left ventricular hypertrophy, and left ventricular dysfunction.[27,28] Our data demonstrated that ADMA is significantly correlated with high sensitivity C-reactive protein and IMT, but not with left ventricular hypertrophy. We hypothesize that this finding was because our study included only patients with normal or mildly impaired renal function and without CVD. Kielstein *et al.* reported that ADMA might be an indicator of incipient renal disease, even when GFR is within the normal range.[11] Our study patients had a significant negative relationship between plasma ADMA levels and eGFR, despite having normal or mildly impaired renal function. Considering these results, elevated plasma ADMA levels in patients with CKD do not seem to be caused only by its accumulation on account of decrease in renal function. Further, our data also indicate that ADMA has a significant association with both eGFR and CFVR, and that plasma

ADMA levels are the highest in patients with both renal and coronary microvascular disease. We therefore speculated that microvascular disease might cause parallel deleterious effects in both kidney and heart, and that ADMA may play an important role in the progression of kidney and heart disease. In our study, ADMA concentration showed a statistically significant independent association with both eGFR and CFVR. These findings strongly suggest that an elevation in plasma ADMA level reflects the degree of microvascular disease in the kidney and heart.

In this study, the significant association of ADMA with eGFR disappeared when age as a factor was included in the multivariate analysis. In agreement with a previous study report,[29] we hypothesized that a possible reason could be that the Cockroft and Gault equation includes age. Therefore, in this study, we excluded the parameter (age) on the additional multivariate analysis with eGFR, and could not completely elucidate the degree of the influence of ADMA on GFR, in relation to age. We therefore chose 20 patients from group A and 20 patients matched for age from group B and compared ADMA levels between the two groups. The result showed that ADMA levels were significantly higher in group B (group A: 0.49 ± 0.07 pg/ml, group B: 0.53 ± 0.07 pg/ml: $P < 0.05$). Furthermore, the relationship between age and ADMA was preserved (data not shown). Another possible explanation is that the influence of age on renal function may be mediated partly by ADMA or an ADMA-related process. It has been demonstrated that microcirculation deteriorates because of vascular structural and functional changes with advancing age.[30,31] It may be further aggravated through an ADMA-related mechanism.

There are several limitations in this study that require to be mentioned. First, there is the possibility that GFR might not reflect intrarenal microvascular damage, because we did not perform renal biopsies. However, the renal etiology of all the patients in this study was considered to be nephrosclerosis. We looked at clinical records and blood examination results, excluded macrohematuria and proteinuria using the urine strip test, and did not detect chronic glomerulonephritis or other renal diseases in our study patients. It has been reported that intrarenal arteriolar lesions are associated with impaired GFR in patients with nephrosclerosis.[32,33] We therefore assumed, for our study group of patients, that eGFR is related to intrarenal microvascular damage. Second, we estimated GFR using the Cockroft and Gault equation and not the inulin-clearance technique. Our method may not reflect an accurate measure of GFR. It is very difficult to perform the GFR measurement using inulin-clearance method for all the subjects, despite almost normal renal function in the clinical setting. However, we intend to perform a further study using the inulin-clearance method to measure GFR, so as to remove this limitation. Third, a cutoff value of CFVR ≤2.0 was originally used for significant coronary artery stenosis; however, it is very difficult to find the cutoff value of CFVR for coronary microvascular disease. We considered that patients with CFVR ≤2.0 despite having no significant coronary artery stenosis have quite severe microvascular disease. We therefore adopted it as a cutoff value for microvascular disease.

Our data indicate that ADMA may be a useful biochemical marker to detect early damage in the kidneys and heart. We speculate that renal and coronary microvascular diseases are closely linked and ADMA may play an important role in the pathogenesis of both diseases. Therefore, we consider that the analysis of ADMA might contribute to detecting the progression of kidney and heart disease in hypertensive patients. Further pharmacological interventional studies are necessary in order to test the potential benefit of ascertaining ADMA levels in these patients.

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Masked Hypertension: Subtypes and Target Organ Damage

YUHEI KAWANO, TAKESHI HORIO, TETSUTARO MATAYOSHI, AND KEI KAMIDE

Division of Hypertension and Nephrology, National Cardiovascular Center, Fujishirodai, Suita, Osaka, Japan

Masked hypertension has been drawing attention recently because this condition is often seen in untreated and treated individuals and is associated with target organ damage and a poor cardiovascular prognosis. Although masked hypertension is defined as normal office blood pressure with elevated ambulatory or home blood pressure, there are several subtypes. Morning hypertension is the most common form of masked hypertension, and is caused by natural circadian variation, evening alcohol consumption, and the use of short-acting antihypertensive drugs. Daytime hypertension may be caused by lifestyle factors such as habitual smoking and mental or physical stress. Nighttime hypertension is seen in various conditions that produce non-dipping status, including a high salt intake, renal dysfunction, obesity, sleep apnea, and autonomic failure. Advanced target organ damage such as increases in the left ventricular mass, carotid artery intima-media thickness, and urinary albumin excretion, is often present both in untreated and treated subjects with masked hypertension. In our study, the presence of the reverse white-coat effect is independently associated with those indices of organ damage among treated hypertensive patients. It is important to identify individuals with masked hypertension, to evaluate them with including the search for the subtype, and to treat each patient appropriately according to the cause of this condition.

Keywords masked hypertension, target organ damage, ambulatory blood pressure monitoring, home blood pressure

Introduction

Masked hypertension, which is also called reverse white-coat hypertension or isolated ambulatory hypertension, has been drawing attention recently (1–3). Masked hypertension is defined as normal office blood pressure (BP) with elevated ambulatory or home BP. Although the term of masked hypertension was originally applied to untreated subjects, this condition is also frequently seen in treated hypertensive

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Address correspondence to Yuhei Kawano, MD, PhD, Division of Hypertension and Nephrology, National Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan; E-mail: ykawano@hsp.ncvc.go.jp

patients. The prevalence of masked hypertension has been reported to be about 10% in normotensive (defined by casual BP) subjects and about 20% among treated hypertensive patients (1,4–6). There is increasing evidence that masked hypertension is associated with advanced target organ damage and a poor cardiovascular prognosis (7–11).

Masked hypertension can be classified into several subtypes according to the pattern of ambulatory BP and underlying mechanisms. These subtypes include morning, daytime, and nighttime hypertension (3). Detecting the subtype and underlying mechanism may be helpful for the appropriate management of each patient with masked hypertension. Regarding the target organ damage in masked hypertension, obtained information may not be enough, especially for treated patients. In this review, we describe the subtypes and organ damage of masked hypertension, including the results of our studies.

Subtypes of Masked Hypertension

Morning Hypertension

Morning hypertension is the most common form of masked hypertension (see Table 1). The circadian rhythm of BP is well known. Usually, BP elevates sharply with waking in the early morning, decreases slightly from the late morning to early afternoon, increases again in the early evening, decreases in the late evening, and then falls largely with sleeping. It has been shown that home BP in the early morning is somewhat higher than that in the late evening (6,12). It is possible that this physiological change in BP causes masked hypertension, if office BP is measured in the late morning or early afternoon in the absence of the white-coat effect. Morning hypertension is also caused by lifestyle-related factors such as habitual alcohol intake. We observed that evening alcohol consumption decreases nighttime BP but increases daytime BP in

Table 1
Subtypes of masked hypertension

Subtypes	Causes	Management
Morning hypertension (morning surge)	Natural circadian rhythm	Alcohol restriction
	Alcohol	Long-acting drug
	Antihypertensive drug (short-acting)	Evening drug administration
		Alpha blockers (evening)
Daytime hypertension (worksite hypertension)	Smoking	Smoking cessation
	Stress (mental, physical)	Stress management
		Beta blockers (morning)
Nighttime hypertension (non-dipper)	Salt, renal dysfunction	Salt restriction
	Obesity, sleep apnea	Weight reduction
	Autonomic failure	Diuretics
		Treatment of sleep apnea

hypertensive patients (12,13). This alcohol-induced BP elevation is most obvious in the early morning.

Morning hypertension is often seen among treated hypertensive patients, particularly in those who are taking short-acting antihypertensive drugs in the morning. Such medication does not maintain the antihypertensive efficacy for 24 hours, resulting in BP elevation in the early morning. The use of long-acting drugs or evening administration of antihypertensive drugs is helpful to control morning hypertension. Because the sympathetic nervous system plays an important role in the morning BP elevation through alpha receptor-mediated vasoconstriction, the administration of alpha blockers in the evening may also be effective to attenuate the morning BP surge (14).

Daytime Hypertension

Daytime hypertension is caused by lifestyle-related factors such as habitual smoking and daily stress (see Table 1). Smoking cigarettes acutely elevates BP, and smokers show a higher daytime BP on a smoking day compared with nonsmokers or a nonsmoking day (15). Mental or physical stress also acts to elevate daytime BP, particularly during working (16). We also observed that daytime BP but not nighttime BP is higher during usual daily life than during a hospital stay in hypertensive patients (17). When habitual smokers or subjects experiencing stress visit clinics, their BP may be normal because they can take a rest without smoking in the waiting room. The cessation of smoking and control of daily stress is recommended for subjects with daytime hypertension. Beta blocker usage may be effective to control stress-related hypertension.

Nighttime Hypertension

Although BP usually falls at night, the nighttime BP dip is blunted or absent in a considerable portion of normotensive and hypertensive subjects. Some individuals show a rise in BP during sleep. This non-dipper pattern is often seen in salt-sensitive subjects on a high-salt diet; patients with renal dysfunction; obese subjects, particularly those with sleep apnea; and patients with autonomic failure; and may cause masked hypertension (see Table 1). It should be mentioned that many non-dippers also show morning hypertension because their BP continues to increase during the night until waking up.

Previous studies by our institute have shown that treatment with a low-salt diet or a diuretic decreases nighttime BP effectively in hypertensive patients (18,19). Weight reduction is recommended for obese subjects. Continuous positive airway pressure treatment is effective to lower nighttime as well as 24-hour BP in patients with sleep apnea (20). It is also important to use long-acting antihypertensive drugs to control nighttime BP.

Identifying the Subtypes

The diagnosis of masked hypertension is obtained by the use of ambulatory BP monitoring (ABPM) or home BP measurement in comparison with office BP. The Japanese guidelines for the management of hypertension (JSH 2004) support the use of ABPM and home BP measurement, particularly for the diagnosis of white-coat hypertension and masked hypertension (21).

To identify the subtypes of masked hypertension, ABPM is superior to home BP measurement because it provides multiple BP readings throughout 24 hours. However, the

application of ABPM to all hypertensive subjects is not practical, and a single ABPM may not be enough to represent the individual's 24-hour BP profile. Self-measurement of BP in the morning and evening at home appears to detect morning hypertension. Daytime hypertension can be detected through additional BP measurement at home or worksite during the daytime. ABPM is particularly suitable for the diagnosis of nighttime hypertension. The detection of nighttime hypertension by home BP measurement is difficult; however, new devices with timers, such as OMRON HEM-747IC, can determine BP during sleep. The widespread application of such devices may easily identify the subtypes of masked hypertension without using ABPM.

Target Organ Damage in Masked Hypertension

Numerous studies have examined the relationship between ambulatory BP or home BP and cardiovascular complications. It has been shown that ambulatory BP and home BP are more closely related to hypertensive organ damage and cardiovascular prognosis than office BP (22–26). Therefore, it is not surprising that subjects with masked hypertension are prone to develop target organ damage.

Untreated Subjects

It has been shown that subjects with masked hypertension have advanced target organ damage and a poor cardiovascular prognosis compared to normotensive subjects. Liu et al. measured target organ abnormality by echocardiography and arterial ultrasonography in untreated subjects with sustained normotension, masked hypertension, and sustained hypertension (27). They demonstrated that left ventricular mass and carotid wall thickness are greater in subjects with masked hypertension compared to those with sustained normotension, and are similar to those with sustained hypertension. Lurbe et al. also showed that young patients with masked hypertension have a higher left ventricular mass index than normotensive subjects (28). It is likely that a majority of masked hypertensives are overlooked because of normal office BP, resulting in the progression of target organ damage.

Treated Patients

Advanced target organ damage is also seen in treated patients with masked hypertension. We determined the left ventricular mass index, carotid artery intima-media thickness, and urinary albumin excretion in 332 treated hypertensive patients (29,30). In our study, all of these indices of target organ damage in patients with masked hypertension were significantly higher than those with controlled hypertension or white coat hypertension, and were even higher than those with sustained hypertension (see Figure 1). Cuspidi et al. examined left ventricular mass index and urinary albumin excretion in treated hypertensive patients at baseline and after an average follow-up of 30 months (31). They observed that these parameters decreased in patients with controlled ambulatory BP but not in those with masked hypertension.

Subtypes and Organ Damage

A number of studies have shown that the non-dipper pattern or the level of nighttime BP is associated with advanced organ damage and a poor prognosis (22–24,32,33). In the PAMELA study, nighttime BP was the best predictor of future cardiovascular death

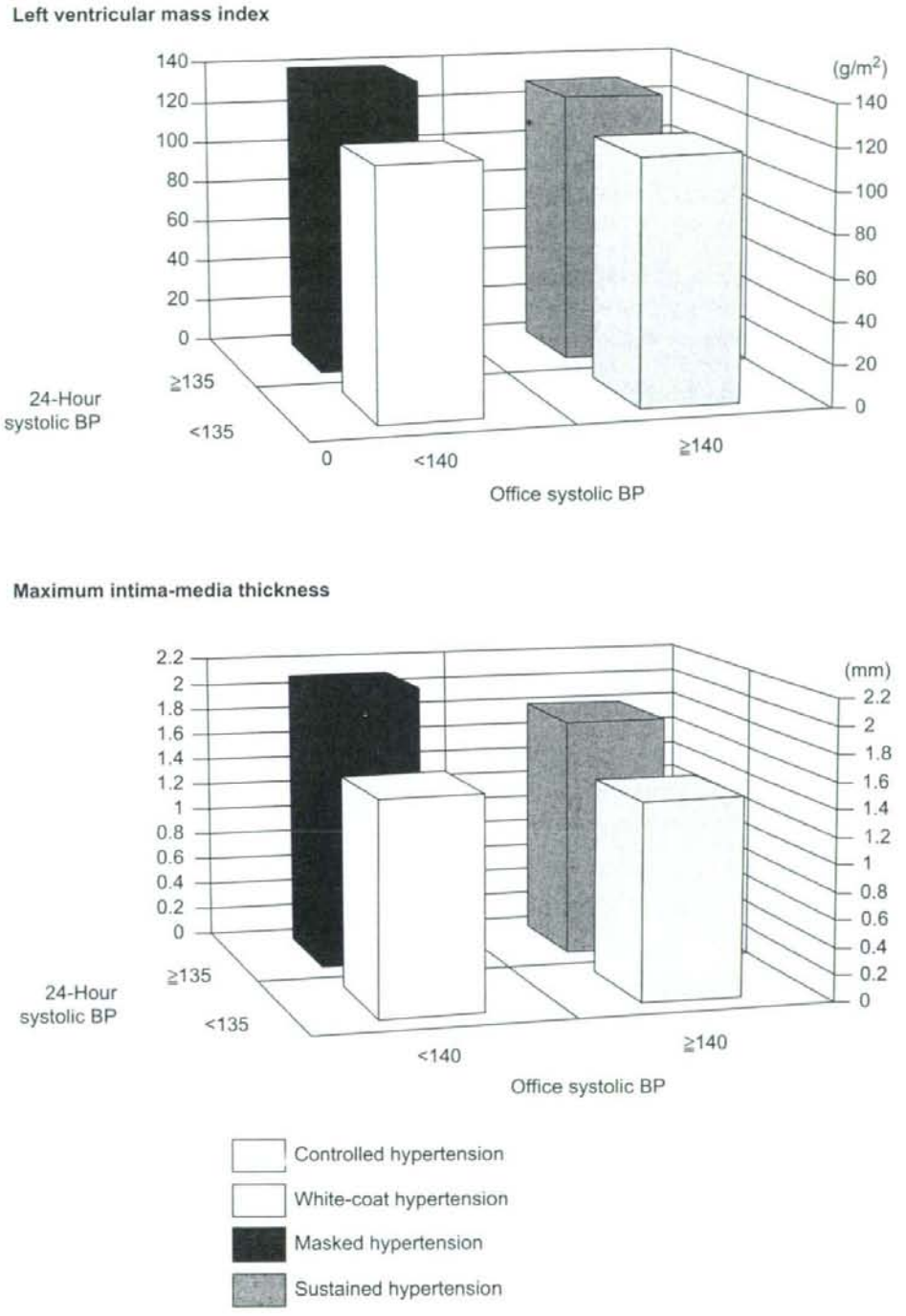

Figure 1. Left ventricular mass index and carotid artery maximum intima-media thickness in treated patients with controlled hypertension, white-coat hypertension, masked hypertension, and sustained hypertension, adopted from (29).

among office BP, home BP, and ambulatory BP parameters (24). Therefore, it is likely that nighttime hypertension is prone to develop organ damage such as left ventricular hypertrophy, carotid atherosclerosis, and impaired renal function.

It is well known that cardiovascular events occur frequently in the early morning when BP increases rapidly. Kario et al. have shown that the morning surge in BP is independently associated with silent and clinical cerebrovascular disease, and morning hypertension is the strongest independent risk factor for stroke in elderly hypertensives (34,35). It is also reported that the morning rise in BP correlates with the left ventricular mass index or hypertrophy in hypertensive patients (36,37), and high morning BP is associated with a loss of functional independence in elderly subjects (38). Therefore, morning hypertension appears to play a role in the target organ damage and cardiovascular events.

The association of daytime BP with organ damage and prognosis is less recognized, although daytime BP is a main determinant of average 24-hour BP. In the PAMELA study, the contribution of daytime BP to cardiovascular mortality was relatively weak compared with nighttime BP (24). However, it has been shown that mental stress is related to the progression of carotid atherosclerosis and cardiovascular mortality (39,40). It is possible that subjects with daytime hypertension are also susceptible to the development of target organ damage.

Conclusion

There are several subtypes of masked hypertension. Morning hypertension is caused by natural circadian variation, evening alcohol consumption, and short-acting antihypertensive drugs. Daytime hypertension may be caused by smoking and stress. Nighttime hypertension is seen in various conditions that lead to a non-dipping status. Advanced target organ damage is often present both in untreated and treated subjects with masked hypertension. All three subtypes of masked hypertension seem to be associated with organ damage, although the relative risk of those subtypes remains to be clarified. It is important to identify individuals with masked hypertension, evaluate them (including identifying the subtype), and treat each patient appropriately according to the cause of this condition.

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Pulmonary venous flow and risk of cardiovascular disease in essential hypertension

Yoshio Iwashima[a], Takeshi Horio[b], Kei Kamide[b], Hiromi Rakugi[a], Toshio Ogihara[a] and Yuhei Kawano[b]

Objective The prognostic significance of the pulmonary venous flow in essential hypertensive patients was investigated.

Methods and results Doppler transthoracic echocardiograms were analyzed in 705 essential hypertensive subjects with no prior cardiovascular disease. At baseline, most subjects had 'normal diastolic function' or 'mild diastolic dysfunction'. During follow-up (mean, 32 months), 56 participants developed cardiovascular disease. Sex-specific median values were used to separate the higher group from the lower group of the peak velocity ratio of the pulmonary venous systolic to diastolic wave (*S/D*) (male <1.51, female <1.66), and of the transmitral velocity ratio of early diastolic to atrial filling (*E/A*) (male <0.84, female <0.82). Kaplan–Meier curves with log-rank tests showed significantly poorer event-free survival rates in the groups with high *S/D* ($P<0.01$) and low *E/A* ($P<0.01$), respectively. In multivariate Cox regression analysis, the *S/D* ratio (HR 1.07 for each 0.1 increase, $P=0.03$) or *E/A* ratio ($P<0.01$) was an independent predictor of cardiovascular disease events. When divided into four groups based on the respective sex-specific median levels of *S/D* in the *E/A* $\geq$ median and *E/A* $<$ median groups, the group with high *S/D* and low *E/A* (*S/D*; male ≥ 1.77, female ≥ 1.81) had a significantly poorer event-free survival rate ($\chi^2=28.06$, $P<0.01$), and the adjusted-hazard ratio by multivariate Cox regression analysis was 2.16 (95% CI; 1.40–3.07, $P<0.01$).

Conclusion Increased *S/D* or decreased *E/A* is associated with an increased cardiovascular disease risk, and the combination of high *S/D* and low *E/A* may be a powerful predictor of cardiovascular disease in essential hypertension. Pulmonary venous flow evaluation may provide clinically important prognostic information in patients with essential hypertension. *J Hypertens* 26:798–805 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Keywords: cardiovascular disease, diastole, echocardiography, hypertension, predictor

Abbreviations: Ad, the duration of atrial filling wave; ANOVA, analysis of variance; ARdur, the duration of flow at atrial contraction; A-velocity, the peak of atrial diastolic phase filling; CI, confidence interval; CVD, cardiovascular disease; DcT, the deceleration time of early diastolic LV filling; E/A, the ratio of peak early to late diastolic filling velocity; E-velocity, the peak of early diastolic phase filling; HR, hazard ratio; LA, left atrial; LAD, left atrial dimension; LV, left ventricular; LVMI, left ventricular mass index; MVF, mitral valve flow; PVa, pulmonary vein atrial reversal; PVd, peak diastolic forward flow velocity; PVF, pulmonary venous flow; PVs, peak systolic forward flow velocity; S/D, the ratio of the pulmonary venous systolic velocity to diastolic velocity

[a]Department of Geriatric Medicine, Osaka University Graduate School of Medicine and [b]Division of Hypertension and Nephrology, Department of Medicine, National Cardiovascular Center, Osaka, Japan

Correspondence to Takeshi Horio, MD, PhD, Division of Hypertension and Nephrology, Department of Medicine, National Cardiovascular Center, 5-7-1, Fujishirodai, Suita, Osaka 565-8565, Japan
Tel: +81 6 6833 5012; fax: +81 6 6872 8091;
e-mail: thorio@ri.ncvc.go.jp

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Introduction

Cardiovascular disease (CVD) such as congestive heart failure, coronary artery disease, and hypertension often leads to systolic and diastolic ventricular dysfunction. It is now recognized that patients with normal systolic function can have marked impairment of diastolic function (isolated diastolic dysfunction) [1]. Comprehensive Doppler echocardiography can now characterize diastolic function directly in addition to measuring systolic function. Doppler left ventricular (LV) diastolic filling indices, especially the ratio of peak early to late diastolic filling velocity (*E/A*), have gained wide acceptance as simple and commonly used indices to assess diastolic dysfunction. Previous studies have reported independent prognostic information on diastolic dysfunction in different clinical settings and populations, such as congestive heart failure [2], myocardial infarction [3,4], and the elderly [5]. Additionally, two recent reports from the PIUMA study [6] and the Strong Heart Study [7] pointed out the prognostic value of the transmitral *E/A* ratio in hypertensive patients and in the general population, respectively.

Noninvasive assessment of pulmonary venous flow (PVF) using pulse-wave Doppler transthoracic echocardiography is proposed as a useful measurement in various disease states [8,9]. Of these measures, the ratio of systolic velocity to diastolic velocity (*S/D*) by assessment of the pulmonary veins is a commonly used index to assess diastolic filling in PVF. Individuals with low *S/D* are considered to have a restrictive filling pattern, whereas those with high *S/D* have impaired early diastolic

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relaxation [8]. Previous studies have explored the prognostic value of PVF by categorizing diastolic dysfunction according to the progression of diastolic dysfunction [10], or in patients with systolic dysfunction [11,12]. These results seem to confirm the association between restrictive LV filling and higher CVD risk. Even in essential hypertensive patients with LV hypertrophy, however, a restrictive LV filling pattern is very uncommon [13], and diastolic dysfunction in hypertensive patients without heart failure is usually characterized by abnormally prolonged relaxation [14,15], that is decreased peak diastolic forward flow velocity (PV_d) and increased peak systolic forward flow velocity (PV_s), resulting in an increased *S/D* ratio [16]. Therefore, this study was undertaken to identify the clinical significance of PVF, in middle-aged and elderly essential hypertensive subjects, to determine its impact on prognosis. In addition, we further examined whether assessment of the PVF velocity pattern adds to the prognostic information provided by *E/A* ratio.

Methods

Study subjects

This study enrolled essential hypertensive patients in normal sinus rhythm, who had good-quality echocardiographic recordings, and monitored for a mean follow-up of 32.0 ± 18.0 months retrospectively. In our laboratory (the National Cardiovascular Center in Osaka, Japan), all hypertensive patients attended the echocardiography laboratory, and echocardiographic data were routinely collected consecutively. Of 865 patients at the time of the baseline examination, we excluded 160 patients (18.5%) for the following reasons: ischemic heart disease, acute coronary syndrome, congestive heart failure [New York Heart Association (NYHA) class II or greater], valvular heart disease, old cerebral infarction, history of transient ischemic attack, secondary hypertension, moderate or severe aortic or mitral regurgitation, heart rate over 100 bpm, or low ejection fraction (< 45%), or those with undetectable PVF throughout the cardiac cycle or absent reversal. After these exclusions, 705 hypertensive patients (350 women) remained. The patients with missing data for PVF were ($n = 126$, 14.6%), on average, obese and had excessive wall motion noise associated with atrial contraction compared with patients for whom PVF was available.

Hypertension was defined as systolic blood pressure of 140 mmHg or over or diastolic blood pressure of 90 mmHg or above on repeated measurements, or receiving antihypertensive treatment. Diabetes mellitus was defined according to the American Diabetes Association criteria [17]. Smoking status was determined by interview, and defined as follows: never-smoker, past-smoker (those who had a history of habitual smoking but had quit), and current-smoker. Ischemic heart disease was defined as a 75% or greater organic stenosis of at least one major coronary artery as confirmed by coronary angiography, or a history of myocardial infarction or percutaneous transluminal coronary angioplasty. All procedures in the present study were carried out in accordance with institutional and national ethical guidelines for human studies. All participants enrolled in this study were Japanese, and all gave informed consent to participate in this study.

Baseline clinical characteristics

After fasting overnight, blood pressure was measured with an appropriate arm cuff and a mercury column sphygmomanometer on the left arm after a resting period of at least 10 min in the supine position. After blood pressure measurement, venous blood sampling from all subjects was performed. Height and body weight were measured, and body mass index was calculated. The following parameters were also determined: total cholesterol, triglycerides, and high-density lipoprotein cholesterol.

Echocardiographic methods and calculation of derived variables

Imaging and Doppler echocardiography were performed in all study participants. Studies were performed using phased-array echocardiography with M-mode, two-dimensional, pulsed, and color-flow Doppler capabilities, as previously described [18,19]. The left atrial dimension (LAD), LV internal dimension and septal and posterior wall thickness were measured at end-diastole and end-systole according to the American Society of Echocardiography recommendations [20]. Color-flow Doppler recordings were used to check for aortic and mitral regurgitation, as previously described [21]. End-diastolic dimensions were used to calculate LV mass by a previously reported formula [22]. LV mass was considered to be an unadjusted variable, and was normalized by body surface area and expressed as LV mass index (LVMI). End- diastole and end-systole LV volumes, calculated by Teichholz's method [23], were used to calculate ejection fraction.

The LV diastolic filling pattern was recorded from the apical transducer position with the subject in the left lateral decubitus position, and with the sample volume situated between the mitral leaflet tips. The leading edge of the transmitral Doppler flow pattern was traced to derive the peak of early diastolic and atrial phase LV filling (*E*-velocity and *A*-velocity, respectively), *E/A* ratio, the deceleration time of early diastolic LV filling (*DcT*), and the duration of atrial filling wave (A_d).

Pulmonary venous flow velocity was also routinely recorded by placing a sample volume about 1 cm into the right superior pulmonary vein [24]. Pulmonary vein systolic (PV_s), diastolic (PV_d), *S/D* ratio, and atrial reversal (PV_a), as well as the duration of flow at atrial contraction (*ARdur*), were recorded. When a biphasic PV_s was detected, the highest peak velocity was used [24]. A_d and *ARdur* were measured as close to the zero baseline as possible from start to termination of flow at atrial

contraction after the P wave on the simultaneously recorded electrocardiogram, and the difference in A_d and *ARdur* was calculated ($ARdur - A_d$). All measurements were performed by one trained investigator who was blinded to the clinical data of the subjects.

Differentiation of diastolic filling patterns

Each participant was placed into one of the following categories of filling pattern after echocardiography: normal filling, $1.0 < E/A$ ratio < 1.5 and $140 < DcT < 220$ ms; impaired relaxation, E/A ratio ≤ 1.0 and $DcT \geq 220$ ms; pseudonormal filling $1.0 < E/A$ ratio < 1.5 and $140 < DcT < 220$ ms, but S/D ratio < 1; restrictive pattern, E/A ratio ≥ 1.5 and $DcT \leq 140$ ms [10,25,26].

Clinical endpoints

For survival analysis, observation began on the day of echocardiography with verified updates through March 2004. All subjects were followed at the National Cardiovascular Center in Osaka, and treated by implementation of standard lifestyle and pharmacologic measures. All participants were periodically referred to our institution for blood pressure control and other diagnostic procedures. The CVD events of interest in this study were myocardial infarction and angina pectoris confirmed by electrocardiographic changes, coronary angiography or myocardial scintigraphy findings, stroke confirmed by clinical symptoms, computed tomography and magnetic resonance angiography or cerebrovascular angiography findings, and congestive heart failure requiring hospitalization. Congestive heart failure was defined by the Framingham Heart Study criteria [27], which require the simultaneous presence of at least two major criteria, or one major criterion in conjunction with two minor criteria [28], and requiring treatment with diuretics, vasodilators, or antihypertensive drugs. The cause of death was classified as CVD if there was sudden death from CVD. All CVD events were determined by an independent review panel of physicians who were unaware of the echocardiographic and clinical findings. Furthermore, patients with clinical evidence of pneumonia or uremia were excluded. For patients who experienced multiple nonfatal episodes of CVD, the analysis included only the first event.

Statistical analysis

Data are presented as mean ± SD for continuous variables and as proportions for categorical variables. The relationships between the S/D ratio and various parameters were assessed using univariate linear regression analysis and Pearson's correlation coefficient. The subjects were divided into two groups according to whether their S/D ratio was below or above the median value by each sex, and then the significance of any differences between groups was evaluated using unpaired t test. Event-free survival analysis was performed using the Kaplan–Meier method to plot the cumulative incidence of CVD according to median value of the baseline S/D ratio or E/A ratio by each sex, and the groups were compared by the Mantel log rank test. Cox proportional hazard analysis was used to examine the association between variables and the cumulative incidence of CVD in crude and multivariate models, after accounting for relevant variables using a P-value of less than 0.05 as the selection criterion. These effects were measured by the hazard ratio (HR) and 95% confidence interval (CI) based on Cox regression models.

We next divided the participants into two groups using the median value of the E/A ratio by each sex, and then stratified the participants into four groups according to the respective sex-specific median values of the S/D ratio in participants with E/A ratio of median or higher or E/A ratio below the median. One-way analysis of variance (ANOVA) with Dunnett's multiple comparison posttest was used to analyze data among the four groups. Event-free survival analysis was performed using the Kaplan–Meier method to plot the cumulative incidence of CVD. The relative risk of CVD events in the Cox proportional hazard analysis was assessed in crude and multivariate models, and the cumulative incidence of CVD was calculated using the group with low S/D and high E/A as a reference for each. A P-value less than 0.05 was considered to be statistically significant. All calculations were performed using a standard statistical package (JMP 4.0; SAS Institute, Cary, North Carolina, USA). The authors had full access to the data and take responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Results

Clinical features

Baseline clinical and biochemical characteristics of the study subjects are listed in Table 1. Overall, most of the total subjects had 'normal diastolic function' or 'mild diastolic dysfunction (impaired relaxation)' [10,25]. LVMI was significantly higher, and the S/D ratio was significantly lower in male than in female participants (LVMI 134.5 ± 32.8 versus 119.4 ± 33.5 g/m^2, S/D ratio 1.57 ± 0.43 versus 1.69 ± 0.39, $P < 0.01$ respectively). There was no significant difference in E/A ratio, DcT, PV_a, or $ARdur - A_d$ between male and female participants. We first examined simple correlations between the S/D ratio and various parameters after dividing the subjects into two groups according to sex. As expected, in both male and female participants, the S/D ratio was significantly associated with age (male $r = 0.45$, female $r = 0.36$, $P < 0.01$, respectively), E/A ratio (male $r = -0.60$, female $r = -0.54$, $P < 0.01$, respectively), DcT (male $r = 0.44$, female $r = 0.39$, $P < 0.01$, respectively), and peak PV_a-velocity (male $r = 0.35$, female $r = 0.23$, $P < 0.01$, respectively), but not LAD (male $r = 0.04$, female $r = 0.08$) and LVMI (male $r = 0.06$, female $r = 0.09$). A significant association between E/A ratio and heart rate ratio was found (male $r = -0.14$, female $r = -0.16$, $P < 0.01$, respectively). The

Table 1 Baseline clinical characteristics of study participants

Variables	Total	*S/D* < median Male < 1.51 Female < 1.66	*S/D* ≥ median Male ≥ 1.51 Female ≥ 1.66
n	705	354	351
Age (years)	61.6 ± 11.9	57.7 ± 13.2	65.4 ± 8.9†
Male (%)	50.2	50.3	50.4
Body mass index (kg/m²)	24.3 ± 3.3	24.2 ± 3.5	24.3 ± 3.2
Duration of hypertension (years)	14.8 ± 10.4	13.7 ± 10.4	15.8 ± 10.4†
Smoking status (%)			
Never/past/current	52.3/30.2/16.6	56.0/25.3/18.7	51.0/34.5†/14.5
Systolic blood pressure (mmHg)	144.2 ± 15.6	142.8 ± 15.1	145.5 ± 16.1*
Diastolic blood pressure (mmHg)	81.5 ± 10.6	81.7 ± 11.1	81.1 ± 10.2
Pulse pressure (mmHg)	62.7 ± 14.0	61.1 ± 13.7	64.3 ± 14.1†
Heart rate (bpm)	66.9 ± 8.8	66.4 ± 9.1	67.2 ± 8.1
Diabetes (%)	23.4	21.5	25.4
Total cholesterol (mmol/l)	5.22 ± 0.82	5.24 ± 0.82	5.21 ± 0.82
Triglycerides (mmol/l)	1.51 ± 1.05	1.52 ± 1.20	1.49 ± 0.88
High-density lipoprotein cholesterol (mmol/l)	1.31 ± 0.40	1.35 ± 0.41	1.27 ± 0.37†
LAD (cm)	3.63 ± 0.47	3.61 ± 0.48	3.65 ± 0.45
LVMI (g/m²)	126.9 ± 34.0	123.1 ± 35.0	130.4 ± 32.5†
Ejection fraction (%)	71.7 ± 7.9	71.1 ± 8.1	72.2 ± 7.7
Peak E-velocity (m/s)	0.70 ± 0.16	0.76 ± 0.16	0.65 ± 0.15†
Peak A-velocity (m/s)	0.83 ± 0.19	0.78 ± 0.17	0.88 ± 0.19†
E/A ratio	0.88 ± 0.27	1.01 ± 0.28	0.76 ± 0.20†
DcT (ms)	232.2 ± 48.6	215.7 ± 39.4	248.6 ± 50.9†
A_d (ms)	145.7 ± 22.0	143.0 ± 21.6	148.4 ± 22.3†
Peak PV_a velocity (m/s)	0.63 ± 0.12	0.59 ± 0.11	0.67 ± 0.12†
Peak PV_d velocity (m/s)	0.40 ± 0.10	0.46 ± 0.11	0.35 ± 0.07†
S/D ratio	1.63 ± 0.42	1.31 ± 0.21	1.95 ± 0.32†
Peak PV_s velocity (m/s)	0.29 ± 0.08	0.27 ± 0.07	0.30 ± 0.09†
ARdur (ms)	116.0 ± 22.7	113.9 ± 23.1	118.3 ± 22.1†
ARdur − A_d (ms)	−29.7 ± 28.5	−29.3 ± 29.4	−30.0 ± 27.7
Diastolic filling patterns (%)			
Normal filling	25.0	37.8	12.0†
Impaired relaxation	73.3	58.8	88.0†
Pseudonormal filling	1.4	2.8	0†
Restrictive filling	0.3	0.6	0
Antihypertensive medication (%)	79.7	76.4	83.2*
Calcium channel blocker	66.5	62.5	70.5*
Beta-blocker	26.9	30.2	23.6*
ACEI or ARB	34.2	33.1	35.3
Diuretic	17.2	14.7	19.7

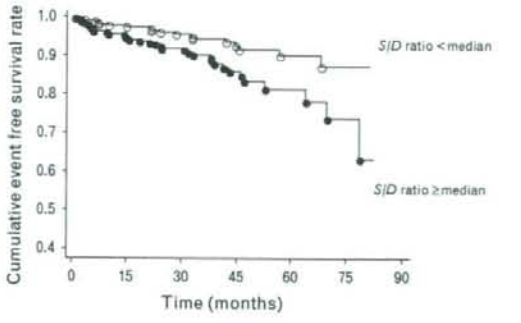
ACEI, angiotensin-converting enzyme inhibitor; A_d, the duration of atrial filling wave; ARB, angiotensin II receptor blocker; *ARdur*, the duration of flow at atrial contraction; A-velocity, the peak of atrial diastolic phase filling; *DcT*, the deceleration time of early diastolic LV filling; *E/A*, the ratio of peak early to late diastolic filling velocity; E-velocity, the peak of early diastolic phase filling; LAD, left atrial dimension; LVMI, left ventricular mass index; PV_a, pulmonary vein atrial reversal; PV_d, peak diastolic forward flow velocity; PV_s, peak systolic forward flow velocity; *S/D*, the ratio of the pulmonary venous systolic velocity to diastolic velocity. Data are mean ± SD or percentage. *$P < 0.05$ and †$P < 0.01$ versus *S/D* < median.

S/D ratio, however, was significantly associated with heart rate in male ($r = 0.11$, $P < 0.05$), but not in female participants ($r = 0.03$, NS).

Predictive value of *E/A* ratio and *S/D* ratio for cardiovascular disease

During the follow-up period, 56 patients (7.9%, 23 females) developed CVD. Specifically, there were 27 patients with nonfatal congestive heart failure, 11 with stroke, six with myocardial infarction, three with angina pectoris, and nine patients died from CVD causes. Among those who developed CVD, 50 patients had impaired relaxation, five with normal diastolic function and one with pseudonormal filling at baseline. The *S/D* ratio was significantly higher and the *E/A* ratio was significantly lower in patients who developed CVD during the follow-up period than in event-free subjects (*S/D* ratio 1.78 ± 0.39 versus 1.62 ± 0.42, *E/A* ratio 0.75 ± 0.18 versus 0.89 ± 0.28, $P < 0.01$, respectively). Because of the sex difference in *S/D* ratios, different median values for men and women were used to separate the high and low *S/D* ratio groups (male < 1.51, female < 1.66). The group with a high *S/D* ratio showed significantly older age, longer duration of hypertension, higher pulse pressure, LVMI, *DcT*, A_d, peak PV_a, and *ARdur*, and lower high-density lipoprotein cholesterol than those with a low *S/D* ratio (Table 1). Life table analysis of CVD throughout the follow-up period in the two groups based on the *S/D* ratio is plotted in Fig. 1. These curves illustrate the significantly poorer event-free survival in the group with a high *S/D* ratio. When the analysis was also performed by applying the sex-specific median values of *E/A* ratios to separate the high group from the low group of *E/A* ratio (male < 0.84, female < 0.82), the group with low *E/A* showed a significantly poorer event-free survival rate (log-rank $\chi^2 = 16.345$, $P < 0.01$). A univariate Cox proportional-hazard model showed that a high *S/D* ratio (HR 1.448, 95% CI 1.10–1.93, $P < 0.01$) and a low *E/A* ratio (HR 1.784, 95% CI 1.34–2.44, $P < 0.01$) were significant predictors of CVD events.

Fig. 1



Cardiovascular event-free survival in two groups with baseline peak velocity ratio of the pulmonary venous systolic to diastolic wave (*S/D*) < or ≥ median value (log-rank $\chi^2 = 7.101$, $P < 0.01$).

Furthermore, even when the *S/D* and *E/A* ratios were included in a univariate model as continuous variables, a significantly higher risk of CVD events was found in these ratios (*S/D* ratio, HR 1.09 for each 0.1 increase, 95% CI 1.03–1.15; *E/A* ratio, HR 0.75 for each 0.1 increase, 95% CI 0.66–0.86, $P < 0.01$ respectively). Other variables in this study that significantly predicted CVD events included LVMI (HR 1.02 for each 1.0 g/m^2 increase, 95% CI 1.01–1.02, $P < 0.01$), LAD (HR 1.01 for each 0.1 cm increase, 95% CI 1.00–1.01, $P < 0.01$), age (HR 1.06 for each 1-year increase, 95% CI 1.03–1.09, $P < 0.01$), diabetes (HR 1.45 for yes, 95% CI 1.10–1.89, $P = 0.010$), and pulse pressure (HR 1.03 for each 1 mmHg increase, 95% CI 1.02–1.05, $P < 0.01$). Sex, body mass index, duration of hypertension, smoking status, systolic and diastolic blood pressure, heart rate, total cholesterol, triglycerides, high-density lipoprotein cholesterol, ejection fraction, and $ARdur - A_d$ were included in the model, but failed to be significant predictors of CVD events. After adjusting for other risk factors (LVMI, age, diabetes, pulse pressure, and LAD) in multivariate Cox regression analysis, independence of the *S/D* (HR 1.07 for each 0.1 increase, 95% CI 1.01–1.14, $P = 0.033$) or *E/A* ratios (HR 0.82 for each 0.1 increase, 95% CI 0.71–0.94, $P < 0.01$) as predictors of CVD events was found. Because heart rate is one of the most important physiological correlates of all parameters of diastolic function, we further performed the multivariate Cox regression analysis after heart rate was added in the model, and found that the *S/D* (HR 1.07 for each 0.1 increase, 95% CI 1.00–1.14, $P = 0.039$) or *E/A* (HR 0.83 for each 0.1 increase, 95% CI 0.71–0.95, $P < 0.01$) ratios were independent predictors of CVD events.

Predictive value of *DcT*, PV_a, and $ARdur - A_d$ for cardiovascular disease

DcT was significantly longer in patients who developed CVD during follow-up (252.4 ± 46.2 versus 230.4 ± 48.4 ms, $P < 0.01$), whereas PV_a and $ARdur - A_d$ were not significantly longer in these patients (PV_a 28.79 ± 7.03 versus 28.69 ± 7.93 m/s, $P = 0.926$; $ARdur - A_d$ −31.61 ± 27.21 versus −29.50 ± 28.62 ms, $P = 0.592$). Even though the prognostic value of *DcT* was significant in a univariate model (HR 1.60 for each 50 ms increase, 95% CI 1.28–1.99, $P < 0.01$), the independence of *DcT* as a predictor of CVD events was lost in the multivariate model (HR 1.29 for each 50 ms increase, 95% CI 0.99–1.66, $P = 0.063$).

Incidence of cardiovascular disease jointly with *S/D* ratio and *E/A* ratio

To assess the combined effects of the *S/D* and *E/A* ratios, we constructed survival curves after dividing the subjects into two groups using the median value of the *E/A* ratio by each sex, and then stratified the subjects into four groups according to the sex-specific median values of the *S/D* ratio in the group with *E/A* ratio of median or above (*S/D* ratio male 1.31, female 1.51) and that with *E/A* ratio under the median (*S/D* ratio male 1.77, female 1.81). As a result, the participants were divided into four groups as follows; low *S/D* and high *E/A*, high *S/D* and high *E/A*, low *S/D* and low *E/A*, and high *S/D* and low *E/A*. The baseline clinical and biochemical characteristics of the study subjects are shown in Table 2. Compared with the group with low *S/D* and high *E/A*, the group with high *S/D* and low *E/A* showed an increased risk of cardiovascular morbidity, such as significantly longer duration of hypertension, higher prevalence of diabetes, higher pulse pressure, and worse dyslipidemia. Life table analyses of CVD throughout the follow-up period according to the four groups of baseline *E/A* and *S/D* ratios are plotted in Fig. 2. These curves illustrate the significantly poorer event-free survival in the group with high *S/D* and low *E/A*. We next performed Cox regression analysis to examine whether the influence of a high *S/D* ratio and low *E/A* ratio on CVD events was independent of other risk factors (Table 3). The risk of CVD was significantly higher in the group with high *S/D* and low *E/A* compared with that in the group with low *S/D* and high *E/A* (HR 2.66). In multivariate Cox regression analysis including LVMI, age, diabetes, pulse pressure, and LAD, the combination of high *S/D* ratio and low *E/A* ratio was an independent predictor of CVD (HR 2.16). Furthermore, even when the group with low *S/D* and low *E/A* was used as a reference, the group with high *S/D* and low *E/A* had a significantly higher risk of CVD events in univariate Cox regression analysis (HR 1.55, 95% CI 1.13–2.19, $P < 0.01$) and in a multivariate model (HR 1.48, 95% CI 1.07–2.10, $P < 0.02$).

In addition, we further performed multivariate Cox regression analysis after heart rate was added in the model, and found that the risk of CVD was significantly higher in the group with high *S/D* and low *E/A* than that in the group with low *S/D* and high *E/A* (HR 2.14, 95% CI 1.38–3.66, $P < 0.01$).