Serum Midkine as a Predictor of Cardiac Events in Patients With Chronic Heart Failure

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ABSTRACT

Background: Midkine, a heparin-binding growth factor, has various functions such as migration of inflammatory cell and anti-apoptotic effect. Invasion of inflammatory cell and cardiomyocyte apoptosis are involved in development and progression of heart failure (HF). However, the relationship between midkine and HF has not been previously examined. Therefore, we examined clinical significance of serum midkine levels to determine the prognosis of HF patients.

Methods and Results: Serum levels of midkine were measured at admission in 216 consecutive patients hospitalized for HF and 60 control subjects. Patients were prospectively followed during a mean follow-up period of 653 ± 375 days with the end points of cardiac death and progressive HF requiring rehospitalization. Serum concentrations of midkine were significantly higher in patients with HF than in controls. Patients with cardiac events had significantly higher concentrations of midkine than those without cardiac events. Kaplan-Meier analysis revealed that cardiac event rates increased markedly as midkine levels rose. Furthermore in the multivariate analysis, after adjustment for age, gender ,and complications, midkine was the independent predictor of cardiac events.

Conclusion: Serum midkine levels are increased in HF patients, and midkine is a novel marker for risk stratifying HF patients. (*J Cardiac Fail 2010;16:308–313*)

Key Words: Prognosis, survival curve, midkine, inflammation.

The 13-kDa midkine is a heparin-binding growth factor which has various functions such as cell growth and survival.[1] Although midkine expression is increased during the midgestational periods in embryogenesis, midkine expression is only detectable in the kidney, lung, thyroid, and intestine in adult periods.[2] However, the tissue stress such as ischemia induces midkine expression in various tissues; for example, the cerebral cortex,[3] vascular endothelial cells,[4] renal tubules,[5] and cardiomyocyte.[6,7] Expression of midkine in heart tissue was increased after myocardial infarction and midkine protected the heart against cardiac remodeling after myocardial infarction by anti-apoptotic effect[6] and angiogenesis,[8] which contributes to prognosis of HF in pressure overload-induced cardiac dysfunction.[9]

HF is characterized by systemic abnormalities, and the cardiomyocyte is continually exposed to various stressors, such as ischemic injury, apoptosis, and various inflammatory reactions by cytokines from inflammatory cells.[10] Several studies have reported that midkine have various functions such as angiogenesis,[11] anti-apoptotic effect,[12] and migration of inflammatory cell.[13] Furthermore, a recent report has demonstrated that midkine plays a regulator of angiotensin II,[14] which is associated with progression of HF via collagen accumulation[15] and myocardial hypertrophy.[16,17] However, the relationship between midkine and pathogenesis of HF has not been previously examined. Therefore, we examined the relation of serum midkine levels to severity and prognosis in HF. The purpose of the

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present study was to clarify relationship between midkine and HF in our institution and to examine whether midkine can reliably risk stratify HF patients with left ventricular (LV) systolic dysfunction or diastolic dysfunction.

Methods

Study Design and Participants

We prospectively studied 216 consecutive patients with HF (132 men, 84 women) who had been admitted for the treatment of worsening HF because of systolic dysfunction or diastolic dysfunction. And 60 age-matched normal subjects (35 men, 25 women; ages 65 ± 12 years) who were hospitalized for cardiac examinations without HF composed the control group. The diagnosis of HF was made by 2 senior cardiologists using the generally accepted Framingham criteria and information including a history of dyspnea and symptomatic exercise intolerance, with signs of pulmonary congestion or peripheral edema or the presence of moist rales on auscultation or documentation of left ventricular enlargement or dysfunction by chest x-ray or echocardiography. Exclusion criteria in this study were patients with clinical or electrocardiographic evidence suggestive of acute coronary syndrome within 3 months preceding admission and those with active hepatic or pulmonary diseases. In addition, we excluded patients and control subjects with malignant diseases and connective tissue diseases that might have influences on serum midkine value.[21,25] Informed consent was obtained from all patients before participation in the study, and the protocol was approved by the human investigations committee of our institution.

Blood samples were obtained at admission from all patients. The glomerular filtration rate (GFR) was estimated using the equation from the abbreviated Modification of Diet in Renal Disease study with the Japanese coefficient, 0.881.[18] We calculated estimate GFR by the following formula: [male] 0.881 × 186 × Age-0.203 × serum creatinine-1.154, [female] 0.881 × 186 × Age-0.203 × Scr-1.154 × 0.742. Transthoracic echocardiography was performed by experienced echocardiographers without knowledge of the biochemical data using an ultrasound instrument (Hewlett-Packard Sonos 7500; Philips Medical Systems, Andover, MA) equipped with a sector transducer (carrier frequency 2.5 or 3.75 MHz) <1 week after admission. Demographics and clinical data, including age, gender, and New York Heart Association (NYHA) functional class at admission, were collected from hospital medical records and patient interviews. Physicians were kept blind to the results of the biochemical markers, and optimal medical therapy that had been instituted was performed independently on the basis of measurements such as improvement in symptoms, physical examination findings, and pulmonary congestion on chest x-ray. The diagnosis of hypertension, diabetes, and dyslipidemia were obtained from medical records or patient histories of currently or previously received medical therapy. Serum midkine

Table 1. Clinical Characteristics of Patients with Heart Failure and Control Subjects

	Control Subjects (n = 60)	HF Patients (n = 216)	*P* Value
Age	65 ± 12	67 ± 13	.2674
Male	35 (58%)	132 (61%)	.6970
NYHA (I/II/III/IV) (53/78/66/19)			
Hypertension	33 (55%)	114 (53%)	.7602
Hyperlipidemia	16 (27%)	45 (21%)	.3354
Diabetes mellitus	13 (22%)	58 (27%)	.4163
Atrial fibrillation	1 (2%)	35 (16%)	.0031
Etiology of heart failure			
Dilated cardiomyopathy		64 (30%)	–
Ischemic heart disease		51 (23%)	–
Hypertensive heart disease		19 (9%)	–
Hypertrophic cardiomyopathy		6 (3%)	–
Valvular heart disease		47 (22%)	–
Others		29 (13%)	–
Laboratory data			
eGFR (mL·min·1.73 m^2)	67.8 ± 12.7	59.9 ± 17.6	.0016
Uric acid (mg/dL)	5.4 ± 1.5	6.1 ± 2.1	.0163
CRP (mg/dL)	0.100, 0.043-0.123	0.200, 0.100-0.600	<.0001
BNP (pg/mL)	19, 9-41	263, 66-755	<.0001
Midkine (pg/mL)	157, 117-226	329, 191-490	<.0001
Log10CRP	−1.105 ± 0.405	−0.586 ± 0.671	<.0001
Log10BNP	1.27 ± 0.52	2.31 ± 0.71	<.0001
Log10Midkine	2.20 ± 0.27	2.49 ± 0.32	<.0001
Echocardiography			
LVEDD (mm)	46 ± 6	55 ± 10	<.0001
LVEF (%)	71 ± 8	49 ± 19	<.0001
Medications at admission			
ACE inhibitors and/or ARBs	14 (24%)	139 (64%)	<.0001
β-blockers	5 (8%)	67 (31%)	.0005
Ca-antagonists	30(51%)	48 (22%)	<.0001
Diuretics	1 (2%)	120 (56%)	<.0001
Spironolactone	0	55 (25%)	<.0001
Digoxin	3 (5%)	52 (24%)	.0012
Statins	16 (27%)	28 (13%)	.0086

NYHA, New York Heart Association; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; BNP, brain natriuretic peptide; LVEDD, left ventricular dimension at end-diastole; LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker.

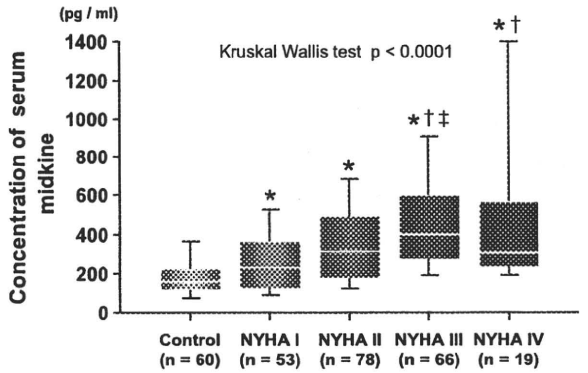

Fig. 1. Association between serum midkine levels and New York Heart Association (NYHA) functional class. Values are presented as the median value (vertical center line), interquartile ranges (up and down sides of box), and the 10% and 90% quartiles (ends of line). *$P < .01$ vs. control subjects; † $P < .01$ vs. NYHA functional Class I; ‡ $P < .05$ vs. NYHA functional Class II.

levels were assessed by a colorimetric enzyme-linked immunosorbent assay (Human Midkine ELISA Development Kit; VERITAS Corporation, Tokyo, Japan). The sensitivity (lower detection limit) was 32 pg/mL.

No patients were lost to follow-up (mean 653 ± 375 days, range 5 to 1095) after admission to Yamagata University Hospital. Patients were prospectively followed until the occurrence of cardiac events in every case. The end points were cardiac death, defined as death from worsening HF or sudden cardiac death, and worsening HF requiring readmission. Sudden cardiac death was defined as death without definite premonitory symptoms or signs and was established by the attending physician. A review of medical records and follow-up telephone interviews to survey cardiac events were conducted by senior cardiologists, who were blinded to blood examination data. Cardiac events were adjudicated using electrocardiography, chest x-rays, autopsy reports, death certificates, and witness statements.

Statistical Analysis

Results are presented as mean ± SD for continuous variables and as percentages of the total number of patients for categorical variables. Skewed variables are presented as the median value (vertical center line), interquartile ranges (up and down sides of box), and the 10% and 90% quartiles (ends of line). Student's unpaired *t*-test and the chi-square test were used for comparisons of continuous and categorical variables between the 2 groups, respectively. If data were not distributed normally, the Mann-Whitney U test was used. Comparisons of data among NYHA functional classes based on midkine levels were performed using the Kruskal-Wallis test. A Cox proportional hazard analysis was performed to determine the independent predictor of cardiac events for the entire population. Significant variables selected in the univariate analysis were entered into the multivariate analysis. The cardiac

Table 2. Univariable and Multivariable Cox Hazard Analyses for Predicting All Cardiac Events in All Heart Failure Patients

	Variables	Hazard Ratio	95% CI of Hazard Ratio	*P* Value
Univariable analysis				
Age (per 5-year increase)	1.209	1.088	−1.345	.0004
Male	1.182	0.717	−1.946	.4880
NYHA	1.779	1.398	−2.264	<.0001
Hypertension	0.634	0.391	−1.029	.1555
Hyperlipidemia	0.980	0.535	−1.795	.4781
Diabetes mellitus	0.822	0.469	−1.441	.5102
Atrial fibrillation	1.104	0.591	−2.062	.5727
Laboratory data				
eGFR (mL/min/1.73 m^2) (per 1 SD decrease)	1.582	1.260	−2.017	.0001
Uric acid (mg/dL) (per 1 SD increase)	1.411	1.128	−1.766	.0026
Log10CRP (per 1 SD increase)	1.448	1.128	−1.858	.0017
Log10BNP (per 1 SD increase)	2.106	1.599	−2.773	<.0001
Log10Midkine (per 1 SD increase)	1.586	1.240	−2.029	<.0001
Echocardiography				
LVEDD (mm) (per 1 SD increase)	1.396	1.073	−1.801	.0119
LVEF (%) (per 1 SD decrease)	1.358	1.039	−1.774	.1492
Medications				
ACE inhibitors and/or ARBs	1.642	0.956	−2.817	.0349
β-blockers	1.376	0.830	−2.283	.1593
Ca-antagonists	0.454	0.225	−0.917	.2057
Diuretics	5.682	2.899	−11.111	<.0001
Spironolactone	1.988	1.214	−3.257	.0115
Digoxin	1.353	0.795	−2.304	.1872
Statins	1.149	0.569	−2.320	.7834
Multivariable analysis				
Log10Midkine (per 1 SD increase)	1.774	1.148	−2.741	.0098

CI, confidence interval.

Other abbreviations as in Table 1.

The multivariate analysis after adjustment for age, gender, New York Heart Association functional class, complications (history of atrial fibrillation, hypertension, diabetes mellitus, hyperlipidemia), etiology of congestive heart failure, laboratory data (estimated glomerular filtration rate, uric acid, common logarithm of C-reactive protein, common logarithm of brain natriuretic peptide), echocardiographic data (left ventricular end-diastolic dimension, left ventricular ejection fraction) and medications.

Table 3. Univariable and Multivariable Cox Hazard Analyses for Predicting Cardiac Deaths in All Heart Failure Patients

	Variables	Hazard ratio	95% CI of Hazard Ratio	*P* Value
Univariable analysis				
Age (per 5-year increase)	1.445	1.193	−1.778	.0002
Male	1.414	0.647	−3.096	.3846
NYHA	2.712	1.799	−4.088	<.0001
Hypertension	0.643	0.312	−1.326	.2320
Hyperlipidemia	0.557	0.194	−1.600	.2767
Diabetes mellitus	1.198	0.548	−2.618	.6516
Atrial fibrillation	1.468	0.630	−3.425	.3733
Laboratory data				
eGFR (mL/min/1.73 m^2) (per 1 SD decrease)	2.025	1.378	−3.035	.0003
Uric acid (mg/dL) (per 1 SD increase)	1.503	1.062	−2.126	.0214
Log10CRP (per 1 SD increase)	1.609	1.093	−2.369	.0159
Log10BNP (per 1 SD increase)	2.303	1.119	−3.621	.0003
Log10Midkine (per 1 SD increase)	2.046	1.399	−2.990	.0002
Echocardiography				
LVEDD (mm) (per 1 SD increase)	1.804	1.219	−2.690	.0032
LVEF (%) (per 1 SD decrease)	1.232	0.828	−1.819	.3122
Medications				
ACE inhibitors and/or ARBs	1.821	0.810	−4.098	.1469
β-blockers	1.553	0.737	−3.279	.2467
Ca-antagonists	0.547	0.208	−1.439	.2213
Diuretics	8.264	2.513	−27.027	.0005
Spironolactone	1.661	0.774	−3.571	.1928
Digoxin	2.387	1.145	−4.975	.0202
Statins	1.048	0.365	−3.003	.9306
Multivariable analysis				
Log10Midkine (per 1 SD increase)	5.777	1.425	−23.42	.0141

CI, confidence interval.
Other abbreviations as in Table 1.
The multivariate analysis after adjustment for age, gender, New York Heart Association functional class, complications (history of atrial fibrillation, hypertension, diabetes mellitus, hyperlipidemia), etiology of congestive heart failure, laboratory data (estimated glomerular filtration rate, uric acid, common logarithm of C-reactive protein, common logarithm of brain natriuretic peptide), echocardiographic data (left ventricular end-diastolic dimension, left ventricular ejection fraction) and medications.

event-free curve was computed according to the Kaplan-Meier method and compared using the log-rank test. All *P* values reported are 2 sided, and a *P* value $< .05$ was considered significant. Statistical analysis was performed with a standard statistical program package (StatView version 5.0; SAS Institute Inc, Cary, NC).

Results

The baseline clinical characteristics of patients with HF and control subjects are listed in Table 1. Uric acid, C-reactive protein (CRP), brain natriuretic peptide (BNP), midkine, and echocardiographic left ventricular end-diastolic dimension (LVEDD) were significantly higher in patients with HF than in control subjects. The estimated GFR and left ventricular ejection fraction (LVEF) were significantly lower in patients with HF than in control subjects. There were no differences in midkine levels between etiologies of HF. No significant correlation was found between serum midkine and BNP values, between serum midkine and LVEF values. As shown in Fig. 1, serum midkine levels were higher in patients with HF than in control subjects and increased with advancing NYHA functional class.

There were 74 cardiac events, including 30 cardiac deaths and 44 readmissions for worsening HF during the follow-up period. The causes of cardiac death were worsening HF in 26 patients and sudden cardiac death in 4 patients. Patients with cardiac events were older and were in higher NYHA functional classes compared with those without cardiac events. Furthermore, the patients with cardiac events showed renal dysfunction, larger LVEDD and higher levels of uric acid, CRP, BNP, and midkine compared with those without cardiac events (midkine: median, interquartile ranges, 377, 269 - 601 pg/mL versus 276, 163 to 451 pg/mL, $P = .0008$).

The ability of prognostic variables to predict cardiac events was examined by the univariate and multivariate Cox proportional hazard analyses. In the univariate analysis, age, NYHA functional class, estimated GFR, uric acid, common logarithm of CRP, common logarithm of BNP, LVEDD, and common logarithm of midkine were associated with subsequent all cardiac events (Table 2). No significant associations were founded between etiology of HF and all cardiac events. Cardiac deaths were also associated with age, NYHA functional class, estimated GFR, uric acid, common logarithm of CRP, common logarithm of BNP, LVEDD, and common logarithm of midkine in the univariate analysis (Table 3). No significant associations were founded between etiology of HF and all cardiac deaths. Furthermore, in the multivariate analysis, after adjustment for age, gender, NYHA functional class,

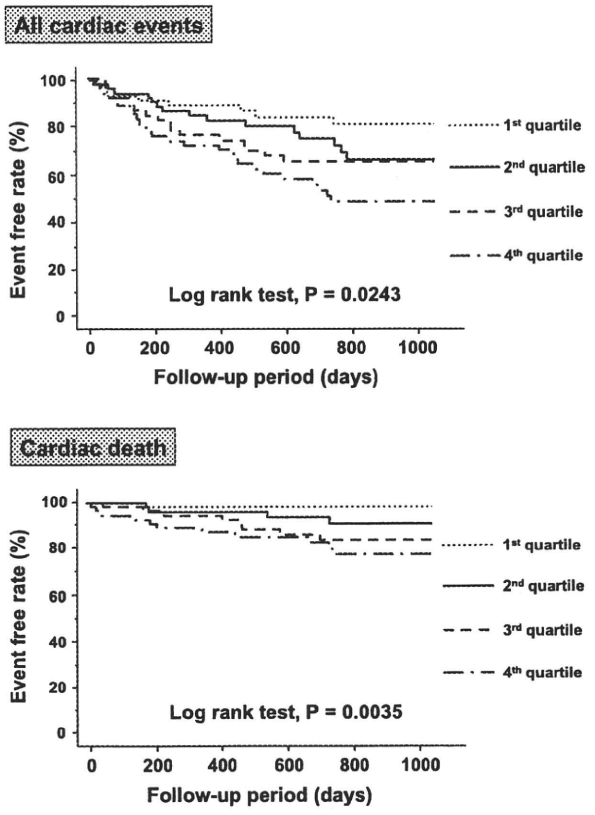

Fig. 2. Kaplan-Meier analysis of cardiac event-free and cardiac death-free in patients with heart failure stratified into 4 groups based on the quartile values of midkine: first quartile (41-190 pg/mL, n = 54), second quartile (191-326 pg/mL, n = 54), third quartile (331-489 pg/mL, n = 54), and fourth quartile (490-2667 pg/mL, n = 54).

complications, etiology of HF, laboratory data, echocardiographic data and medications, common logarithm of midkine was the independent predictor of cardiac events and cardiac deaths (Tables 2, 3).

Next, we classified all patients with HF into 4 groups based on the quartile values of midkine: first quartile (41 to 190 pg/mL, n = 54), second quartile (191 to 330 pg/mL, n = 54), third quartile (331 to 489 pg/mL, n = 54), fourth quartile (490 to 2667 pg/mL, n = 54). As shown in Fig. 2, Kaplan-Meier analysis demonstrated that cardiac event rates were significantly increased from the first to the fourth quartile. Furthermore, cardiac death rates were also significantly increased from the first to the fourth quartile.

Discussion

In the present study, we showed the following findings: (1) serum midkine levels were significantly elevated in patients with HF compared with healthy control subjects; (2) midkine levels increased with advancing NYHA functional class; (3) midkine levels were higher in patients with cardiac events compared with those without cardiac events; (4) multivariate Cox proportional hazard analysis demonstrated that midkine levels were the independent factor to predict adverse clinical outcomes in patients with HF; and (5) Kaplan-Meier analysis showed that cardiac event rates increased markedly as midkine levels rose.

Previous reports have shown the relationship between midkine expression and ischemia/reperfusion induced cardiac damage in vivo study[6-8]; however, clinical significance of midkine levels has not been examined in HF. In the present study, we demonstrated that serum levels of midkine were higher in patients with HF than in control subjects and increased with advancing NYHA functional class. Although the detailed mechanism of midkine overexpression and release in HF patient has been unknown to date, cardiac damage and stress in failing heart might enhance release of midkine into the circulation from heart tissue. Importantly, midkine activates migration of inflammatory cells such as macrophages and neutrophils[13] and emphasize inflammatory response, which play an important role in the development and progression of cardiac abnormalities in HF.[10,19,20] It has been demonstrated that peripheral blood levels of midkine are associated with several inflammatory disorders.[21,22] Maruyama et al revealed that serum and synovial fluid midkine levels were increased in patients with rheumatoid arthritis, and were correlated with severity of rheumatoid arthritis.[21] Taken together, the increased circulating midkine levels might reflect underlying cardiac stress levels.

Recently, there has been considerable interest in the pivotal role of midkine as a regulator of angiotensin II,[14] which is the most physiologically important product of the renin-angiotensin system (RAS) cascade and synthesized in the diseased heart.[23] The circulating midkine might upregulate RAS cascade, which contributes to progression of HF via collagen accumulation[15] and myocardial hypertrophy.[16,17] Recently, Hobo et al have clarified the important crosstalk between regulation of midkine levels and activity of the RAS using in vivo study.[14] Although we could not evaluate the levels of RAS activity in this study population, RAS-related signals might be emphasized by circulating midkine in patient with HF.

Broad biological effects of midkine have been widely evaluated in vivo and vitro study; however, potential mechanisms of midkine in terms of cell survival or organ damage have been clearly undefined yet. There is increasing evidence that midkine contributes to disease progression, as discussed previously. On the contrary, protective effect of midkine against ischemia/reperfusion has also reported.[7,8] Horiba et al have revealed that midkine deficiency increased ischemia/reperfusion injury through Bcl-2 suppression.[6] Because pathogenesis of HF is associated with many mechanisms including protein synthesis, hypertrophy, angiogenesis, inflammation, and apoptosis,[9,10,17,19,20,23] the effect of midkine on disease severity might depend on environmental state of organ specific situation. In accordance with our suspect, Sato et al have shown that suppression of

midkine abolished ischemia/reperfusion-induced organ damage.[24]

Potential limitation of our study is the lack of specificity of high midkine levels. In the present study, we excluded patients diagnosed as malignant diseases[25] or connective tissue disease[21]; however, recent studies revealed that midkine levels were elevated in a wide variety of conditions, including not only malignant diseases and connective tissue disease but inflammatory enteritis,[26,27] short stature, diabetes, obesity, and cleft lip and palate.[28] Thus, it is possible that elevated midkine levels in patients with HF were a completely nonspecific finding. However, Kaplan-Meyer analysis demonstrated that both rates of cardiac death, defined as death from worsening HF or sudden cardiac death, and worsening HF requiring readmission were increased from the first to the fourth quartile, suggesting levels of midkine in HF patients mainly reflect myocardial damage or chronic heart failure processes.

In conclusion, serum levels of midkine were increased in patients with HF and were independently associated with an increased risk for cardiac events. These data indicate that the serum midkine provides useful prognostic information for clinical outcome in patients with HF.

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Trend of Westernization of Etiology and Clinical Characteristics of Heart Failure Patients in Japan

— First Report From the CHART-2 Study —

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Background: Hospitalization due to acute heart failure syndrome (AHFS) is an indicator of worsened prognosis for patients with cardiovascular disease (CVD). The Chronic Heart Failure Analysis and Registry in the Tohoku District 2 (CHART-2) Study was designed to elucidate characteristics and prognosis of patients at high risk for CVD progression due to AHFS.

Methods and Results: The CHART-2 Study is a prospective observational multicenter cohort study. Patients with overt HF, structural cardiac disorder but without HF, or with coronary artery disease (CAD) have been consecutively enrolled from October 2006. As of March 2010, a total of 10,219 patients have been recruited, making the Study the largest multicenter prospective cohort of HF patients in Japan. The mean patient age was 68.2± 12.3 years and male patients accounted for 69.8%. Overt HF was observed in 46.3% of patients; and 53.7% did not have HF but were at high risk for AHFS. As HF stage progressed, the prognostic risks (eg, chronic kidney disease, reduced ejection fraction, and increased B-type natriuretic peptide level) became more prominent. Compared with the previous CHART-1 study, the prevalence of ischemic etiology and risk factors (hypertension, diabetes) have increased, as in Western studies.

Conclusions: This first report demonstrates the trend of westernization of ischemic etiology and clinical characteristics of HF patients in Japan, indicating the importance of appropriate management and prevention of CAD to prevent AHFS. (*Circ J* 2011; **75:** 823–833)

Key Words: Coronary artery disease; Heart failure; Prognosis; Risk factors

Cardiovascular disease (CVD) is the leading cause of death in most developed countries.[1] Furthermore, many developing countries are now catching up with regard to this trend.[1] Heart failure (HF) is the end-stage of CVD and is becoming more common all over the world because of the westernization of lifestyle, the rapid aging of the population, and the increased number of survivors of serious cardiovascular illness due to recent advances in medical and surgical treatment.[2,3] We previously performed a multicenter prospective cohort study of HF patients (Chronic Heart Failure Analysis and Registry in the Tohoku District 1 Study: CHART-1) from February 2000 to December 2005 (n=1,278). The CHART-1 Study found that HF patients were also prevalent in Japan and that the prognosis was similarly poor compared with that in Western countries.[4,5] The most prevalent etiology of HF in the CHART-1 Study was non-ischemic cardiomyopathy (28.6%), and coronary artery disease (CAD) accounted for only 25.4% of the total HF patients, which was considerably low compared with a Western HF study.[3] Hospitalization due to the onset of acute heart failure syndrome (AHFS) is a key event in the disease progression of HF and CVD. Thus, it is important to avoid the decompensation of chronic HF and prevent de novo development of congestive HF in CVD patients in order to improve their long-term quality of life.[6,7] Western studies reported that the most frequent etiology of AHFS was ischemic in origin,[8,9] but the characteristics of such patients at high risk in Japan and the type of pathophysiologic derangement that causes decompensation from stable HF remain uncertain. Furthermore, although a large number of studies have shown that most

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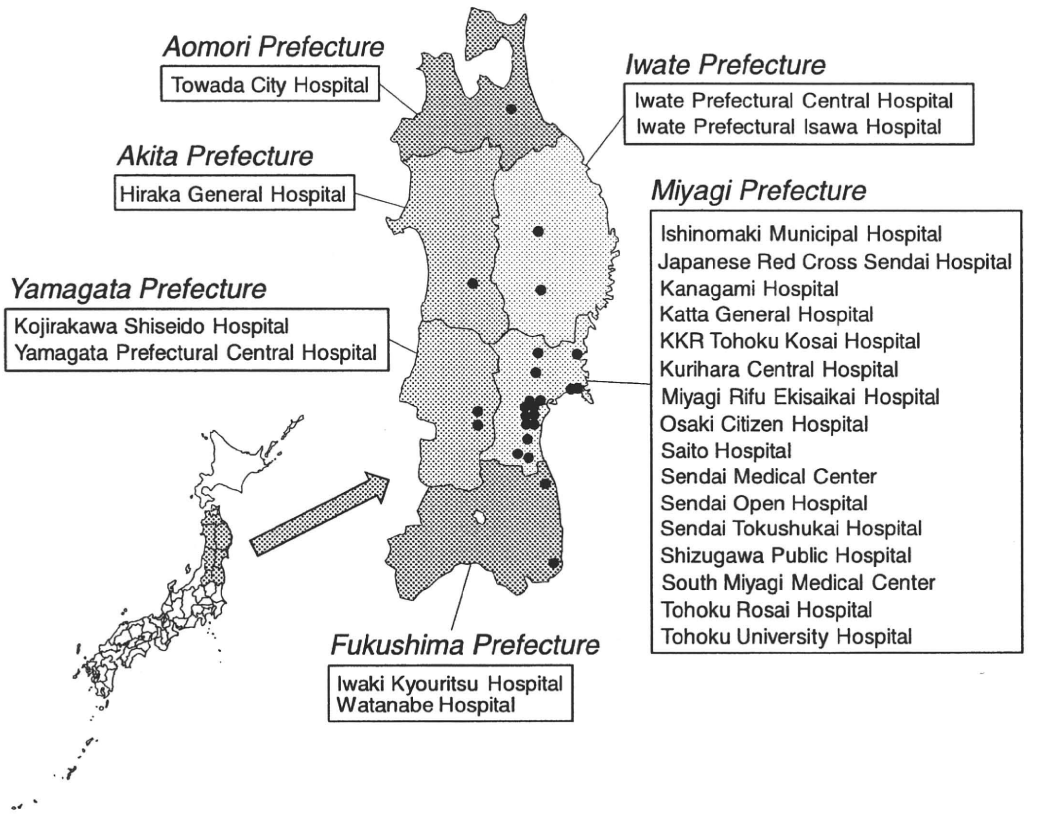

Figure 1. Map of the distribution of 24 collaborating hospitals in the Tohoku district.

patients with HF have preserved ejection fraction (pEF), as observed in the outpatient clinic, there is no evidence-based treatment guideline for such patients.[10,11] Patients with HFpEF are characterized as being more likely to be elderly, to be female and to have more comorbidities (eg, chronic kidney disease [CKD], chronic obstructive pulmonary disease, history of stroke and malignancy). Indeed, the pathophysiology of HFpEF is considered to be more closely related to those extracardiac factors compared with HF with reduced EF (HFrEF).[12,13] Another factor that is associated with the acceleration of the progression of CVD is the lower rate of achievement of clinical guideline-recommended treatment goals.[14,15] We need to regularly evaluate the penetration rate of evidence-based treatment and emphasize the appropriate adherence to the guidelines by physicians and patients.

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Thus, we started a large-scale multicenter prospective cohort study, named the Chronic Heart Failure Analysis and Registry in the Tohoku District 2 (CHART-2) Study, of consecutively enrolled patients at high risk for disease progression of CVD or HF due to the development of AHFS. In this first report of the CHART-2 Study, we examined the trend of etiology of HF patients and their characteristics as compared with the CHART-1 Study.[4,5]

Methods

Study Design and Specific Objectives

The CHART-2 Study is a prospective observational multicenter cohort study to identify the characteristics, mortality and prognostic risks of patients with overt HF and patients without HF but who are at high risk for disease progression of CVD. The purpose of the study was to evaluate the following: (1) characteristics of patients with overt HF and the associated prognostic risks; (2) characteristics of patients at risk for HF and the factors associated with CVD progression; (3) factors associated with the development of AHFS; (4) prevalence and prognostic impact of metabolic syndrome (MetS) in patients with overt HF; (5) the association between MetS and the development of AHFS; (6) the prevalence and prognostic impact of malignancy in patients with CVD; and (7) the prevalence of patients needing home nursing care and the characteristics of bedridden patients with CVD.

Information Disclosure

Rationale, design, and objectives of the CHART-2 Study were registered in clinicaltrials.gov (NCT00418041) and the University Hospital Medical Information Network (UMIN000000562) on the commencement of patient enrollment, and were updated instantly when modifications were made. Detailed information on the CHART-2 Study is available to the public on the Tohoku Heart Failure Association website (http://tohoku.cardiovascular-medicine.jp).

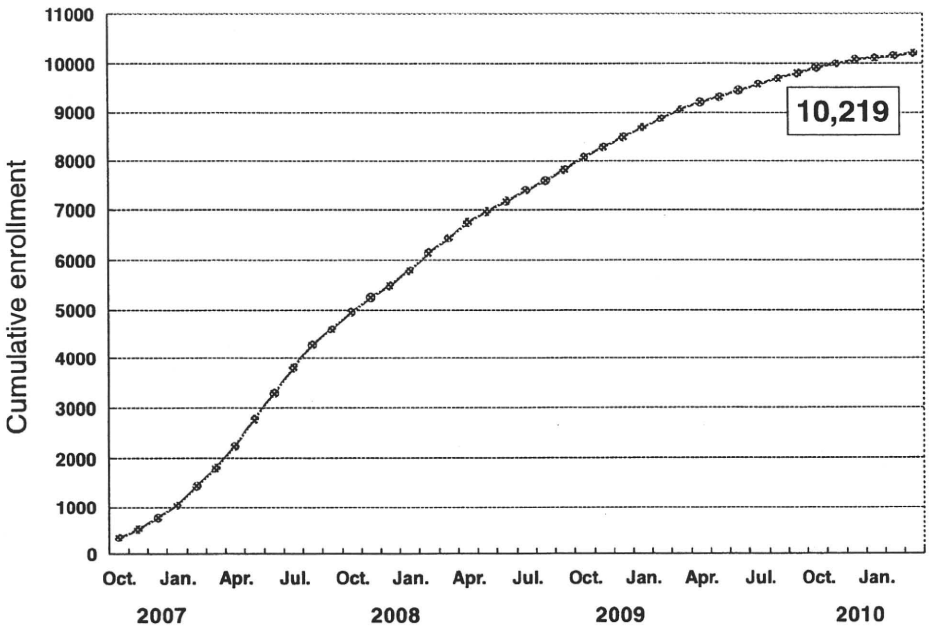

Figure 2. CHART-2 Study cumulative enrollment, showing a total of 10,219 registrations.

Site Selection

A total of 24 institutions, located in the Tohoku district, participated in the CHART-2 Study (Figure 1). A society was organized for the collaborating members and institutions, named the Tohoku Heart Failure Association, before the commencement of the study. The Tohoku district is located in the north-east of Japan and is composed of 6 prefectures, which include approximately 9.8 million individuals in total. The participating institutes and all collaborating members are listed in Appendix 1. Of 24 collaborating institutions, 15 hospitals also participated in the CHART-1 Study (Appendix 1). Patients enrolled in those 15 institutions accounted for 74.0% and 75.8% of the total subjects included in the CHART-1 and CHART-2 Studies, respectively.

Study Group

Stable patients were eligible for enrollment in the CHART-2 Study if they were aged ≥20 years with CAD or were in stage B, C or D defined according to the Guidelines for the Diagnosis and Management of Heart Failure in Adults authorized by the American College of Cardiology Foundation/American Heart Association.[2] In the present cohort study, patients who were asymptomatic but who had structural heart disease and/or impaired left ventricular (LV) function were categorized as being in stage B (Appendix 2). Stage C was defined as current or past symptoms of HF associated with underlying structural heart disease; and stage D was defined as refractory HF in which specialized and advanced treatment strategies were indicated.[2] HF was diagnosed according to the criteria of the Framingham Heart Study.[16] Patients who had been enrolled in the CHART-1 Study were not included in the CHART-2 Study. There were no other exclusion criteria in the present study. The CHART-2 Study was approved by the local ethics committee in each institution. Significant CAD was defined as either organic CAD requiring revascularization or vasospastic angina documented on electrocardiography or angiography. Eligible patients were consecutively recruited after written informed consent was obtained.

Data Collection and Processing

Eight clinical research coordinators (CRC) who belonged to the head office of the CHART-2 Study at Tohoku University visited collaborating hospitals regularly. They fully assisted attending physicians in registration, including candidate screening, explanation of the study design, obtainment of written informed consent, and data extraction from medical charts. Data were entered using a Web-based data collecting system (newly developed by Fujitsu Tohoku Systems) by CRC and trained keypunchers. An identification number was assigned to each enrolled patient and personal information was completely excluded. Data were recorded with regard to demographics, medical history, smoking history, alcohol use, family history of CVD, comorbidities for cardiovascular risks, laboratory findings, echocardiography reports, findings of coronary angiography, previous surgical treatments, and medications at entry. Anemia was defined as hemoglobin <12 g/dl in women and <13 g/dl in men, following the World Health Organization definition.[17] CKD was diagnosed when estimated glomerular filtration rate was $<60\,ml \cdot min^{-1} \cdot 1.73\,m^{-2}$, which was calculated using the formula for Japanese individuals.[18] MetS was defined according to the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome.[19]

Follow-up Survey and Study Outcomes

All follow-up data and events are surveyed once a year during the study period. Collected data were monitored at least twice yearly. Planned completion of the follow-up period is March 2013. Several predefined outcomes including development of AHFS, mortality and other events worsening HF status will be collected in the CHART-2 Study.

Table 1. Baseline Characteristics of the CHART-1 and CHART-2 Patients vs. HF Stage

	CHART-1 (Stage C/D, 2004)	P value*	CHART-2 (2010)				
			Total	Stage B or CAD without HF	Stage C	Stage D	P value**
No. patients	1,078		10,219	5,484 (53.7)	4,640 (45.4)	95 (0.9)	
Age (years), mean±SD	68.7±13.4	0.8	68.2±12.3	67.6±12.2	68.8±12.3	74.2±12.5	<0.001
<40 (%)	3.5	0.4	3.1	3.4	2.7	1.1	<0.001
40–64 (%)	29.2		29.0	29.6	28.5	21.1	
65–74 (%)	31.7		33.7	35.6	31.8	22.1	
≥75 (%)	35.6		34.2	31.4	37.0	55.8	
Male (%)	64.5	0.01	69.8	71.0	68.5	64.2	0.01
Outpatients (%)	NA	NA	79.5	80.3	79.0	60.6	<0.001
NYHA functional class (%)							
I	6.7	<0.001	47.4	68.3	23.4	9.5	<0.001
II	72.9		46.9	30.8	66.5	21.1	
III	19.5		5.3	0.8	9.8	43.2	
IV	0.9		0.4	0.0	0.3	26.3	
Blood pressure (mmHg), mean±SD							
Systolic	126.3±19.1	0.9	128.3±18.6	130.1±17.9	126.4±19.1	119.1±22.4	<0.001
Diastolic	71.5±11.0	0.08	73.5±11.8	74.5±11.5	72.3±11.9	69.2±13.2	<0.001
Heart rate (/min), mean±SD	74.7±14.3	<0.001	71.0±14.1	69.7±13.2	72.4±15.0	72.7±14.5	<0.001
BMI (kg/m²), mean±SD	23.0±3.7	<0.001	24.0±3.6	24.2±3.5	23.8±3.9	21.6±3.4	<0.001
<18.5 (%)	9.2	<0.001	6.6	4.8	8.3	20.0	<0.001
18.5–22.9 (%)	42.9		33.9	32.3	35.5	47.4	
23.0–24.9 (%)	20.6		23.5	25.0	21.9	21.1	
25.0–29.9 (%)	23.5		30.7	33.0	28.4	9.5	
≥30 (%)	3.7		5.3	4.9	5.9	2.1	
Waist circumference (cm), mean±SD	NA	NA	85.9±9.9	86.6±9.5	85.3±10.3	81.4±8.5	<0.001
Male	NA	NA	87.2±9.0	87.7±8.8	86.6±9.2	82.6±8.1	<0.001
Female	NA	NA	83.1±11.2	83.9±10.4	82.4±11.9	79.2±9.0	<0.001
Smoking (%)							
Never	NA	NA	52.7	51.7	53.7	63.2	0.052
Current	NA	NA	18.2	18.3	18.3	14.9	
Former	NA	NA	29.1	30.1	28.0	21.8	
Alcohol (%)							
Never	NA	NA	49.8	48.5	51.1	60.5	<0.001
Regular	NA	NA	27.7	30.0	25.1	19.8	
Chance	NA	NA	14.7	14.4	15.2	4.7	
Former	NA	NA	7.8	7.1	8.5	15.1	
Cardiothoracic ratio (%), mean±SD	NA	NA	52.1±6.5	50.7±5.8	53.6±6.9	57.0±8.1	<0.001
Laboratory findings, mean±SD							
Hemoglobin (g/dl)	13.0±2.2	0.007	13.4±2.0	13.6±1.8	13.2±2.2	12.0±2.5	<0.001
eGFR (ml·min^{-1}·1.73m^{-2})	60.9±30.7	0.9	64.5±22.6	67.5±21.2	61.1±23.5	53.2±29.6	<0.001
HDL-cholesterol (mg/dl)	NA	NA	52.2±15.4	52.9±15.3	51.5±15.6	50.8±14.9	<0.001
LDL-cholesterol (mg/dl)	NA	NA	105.7±30.0	106.3±29.4	105.3±30.9	93.7±26.2	0.001
Fast plasma glucose (mg/dl)	NA	NA	116.7±36.8	115.6±35.4	118.0±38.1	115.6±49.3	0.01
Hemoglobin A_{1c} (%)	NA	NA	5.8±1.0	5.8±0.9	5.9±1.0	5.8±1.1	<0.001
Uric acid (mg/dl)	NA	NA	5.9±1.6	5.7±1.5	6.2±1.8	6.6±2.2	<0.001
Other intervention							
CRT/ICD (%)	1.5	0.002	1.9	0.9	2.9	15.8	<0.001
Heart surgery (%)	NA	NA	14.4	10.9	18.6	18.9	<0.001
PCI (%)	NA	NA	36.8	40.6	32.6	26.3	<0.001
BNP (pg/ml), mean±SD	273.0±352.6	<0.001	145.4±249.3	97.6±188.1	191.4±283.5	454.3±555.6	<0.001
Urine albumin (mg/g·Cre), mean±SD	NA	NA	129.6±476.7	106.5±429.9	157.6±530.1	180.9±330.0	0.001

HF, heart failure; CAD, coronary artery disease; NYHA, New York Heart Association; BMI, body mass index; NA, not applicable; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRT, cardiac resynchronization therapy; ICD, implantable cardioverter defibrillator; PCI, percutaneous coronary intervention; BNP, B-type natriuretic peptide; Cre, creatinine.
*Comparison of stage C/D patients in the CHART-1 Study with those in the CHART-2 Study. **Comparison of stage B/CAD, stage C, and stage D in the CHART-2 Study.

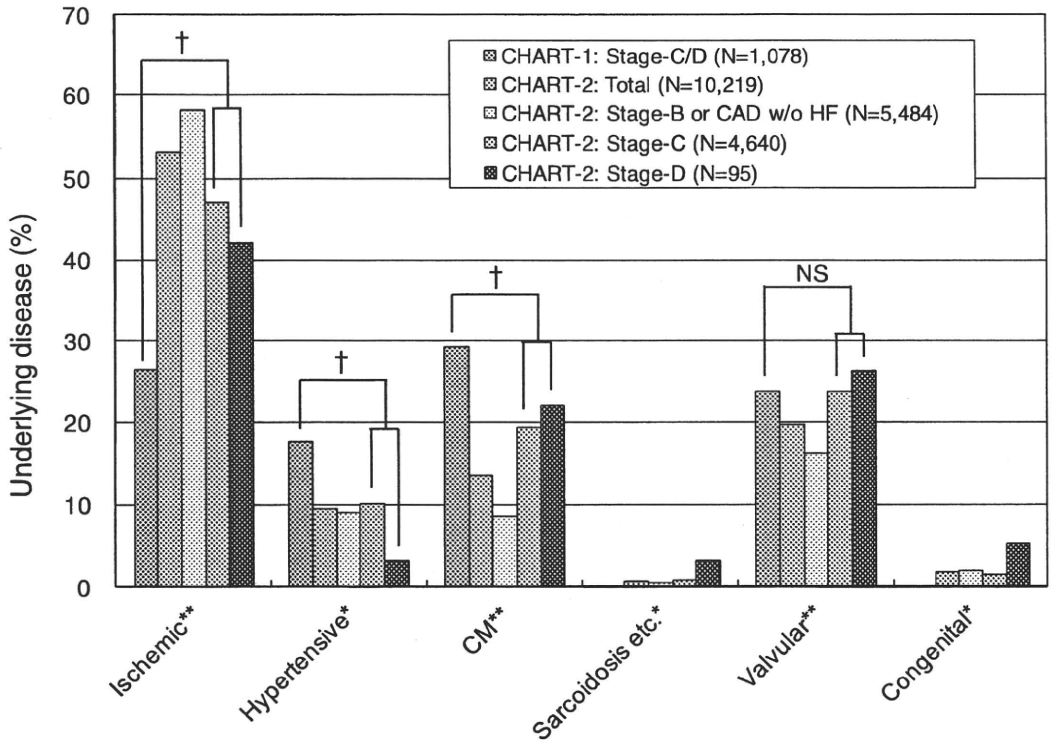

Figure 3. Underlying disease of the CHART-1 and the CHART-2 patients. CAD, coronary artery disease; CM, cardiomyopathy; HF, heart failure; w/o, without. *P<0.05 and **P<0.001 between patients in stage B/CAD, stage C, and stage D in the CHART-2 Study. †P<0.001 between stage C/D patients in the CHART-1 Study and those in the CHART-2 Study.

Statistical Analysis

We divided the study patients into 3 groups: patients with CAD but without HF or who were in stage B; those in stage C; and those in stage D. Comparisons of data between the 3 groups were performed using ANOVA test for continuous variables and chi-squared test for dichotomous variables. Continuous data are given as mean±SD. In order to elucidate the trend of HF in Japan, we selected overt HF patients from the CHART-1 Study (n=1,078, 84.4% of the total cohort), who were categorized as being in stages C or D. We then compared the characteristics of the stage C/D patients in the CHART-1 Study with those in the CHART-2 Study.[3,5] All statistical analyses were performed using IBM SPSS Statistics 19.0, and statistical significance was defined as 2-sided P<0.05.

Results

The enrollment of patients in the CHART-2 Study was started in October 2006. The registration period was prolonged once to achieve the target enrollment number. As of March 2010, a total of 10,219 patients have been enrolled at 24 institutions and the recruitment of patients has been closed, making the Study the largest multicenter prospective cohort of HF patients in Japan (Figure 2).

Clinical Profiles of the CHART-2 Patients at Registration

The mean age of the total study population was 68.2±12.3 years. Male patients accounted for 69.8%, and 79.5% of the total subjects were outpatients. In the present study, 5,484 patients (53.7%) did not have HF but had CAD or cardiac structural disorder. The stage C group included 4,640 patients and accounted for 45.4% of the entire cohort, while 95 patients (0.9%) were classified as being in stage D. Baseline characteristics of the CHART-1 stage C/D patients and the total CHART-2 subjects are given in Table 1. These data including age, sex, vital signs, HF symptoms, anthropometric data, history of smoking, alcohol use, and laboratory findings illustrate the difference in patient characteristics between the 2 studies performed at approximately 6-year intervals. Etiology, comorbidity, medication and echocardiographic findings at registry in the 2 studies are also given in Figures 3–6, respectively.

Baseline Characteristics and Different Clinical Profile vs. HF Stage

Clinical profiles of the CHART-2 patients were considerably different between the 3 HF stages. Mean age increased and HF symptoms became more severe as HF stage progressed (Table 1). Mean systolic/diastolic blood pressure at registration was 128.3/73.5 mmHg and decreased significantly with progression of HF stage. Mean body mass index was 24.0±3.6 kg/m^2 and mean waist circumference was 87.2±9.0 cm in men and 83.1±11.2 cm in women. The factors for obesity status significantly decreased with HF severity (Table 1). MetS as defined by the Japanese criteria was also significantly less frequent in patients in stage C or D compared with those in stage B or those who had CAD but without HF

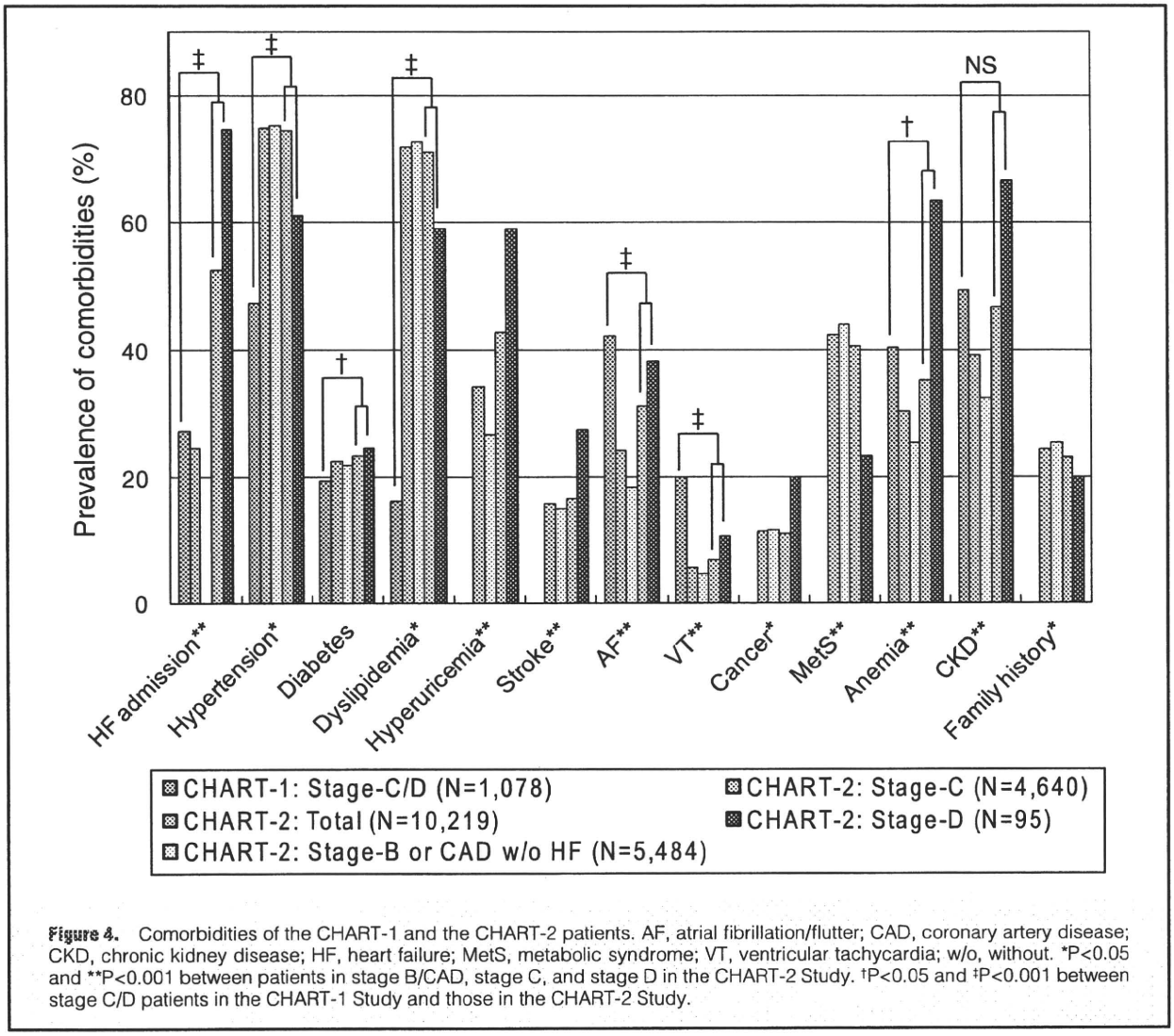

Figure 4. Comorbidities of the CHART-1 and the CHART-2 patients. AF, atrial fibrillation/flutter; CAD, coronary artery disease; CKD, chronic kidney disease; HF, heart failure; MetS, metabolic syndrome; VT, ventricular tachycardia; w/o, without. *P<0.05 and **P<0.001 between patients in stage B/CAD, stage C, and stage D in the CHART-2 Study. †P<0.05 and ‡P<0.001 between stage C/D patients in the CHART-1 Study and those in the CHART-2 Study.

(**Figure 4**). Approximately 18% of patients with CVD had a smoking habit and approximately 28% of the total patients were regular alcohol drinkers (**Table 1**).

Etiology of CVD in the CHART-2 patients is shown in **Figure 3**. CAD was the most prevalent etiology of CVD (53.1%), and approximately 20% of patients had valvular abnormalities as a cause of CVD. Cardiomyopathy accounted for 13.6% of the CHART-2 patients, and the prevalence increased as HF stage progressed. Myocardial diseases due to sarcoidosis or amyloidosis were observed in 0.7% of the total population.

Figure 4 illustrates comorbidities of the CHART-2 patients. The proportion of patients with a history of hospitalization for HF was 52.5% in stage C and 74.7% in stage D. Histories of hypertension or dyslipidemia were very common (74.9% and 71.8%), and diabetes was observed in 22.5% of the total population. Approximately 12% of patients had malignant neoplasm at enrollment. The prevalence of CKD increased significantly as HF stage progressed, accompanied by an increased percentage of patients with anemia and elevated urine albumin excretion (**Table 1**). Patients with overt HF, who were categorized in stages C or D, were also characterized by higher prevalence of atrial fibrillation/flutter, ventricular tachycardia and a history of stroke.

Heart surgery and percutaneous coronary intervention were performed in 14.4% and in 36.8% of the study population, respectively. The rates of use of implantable cardioverter defibrillator and cardiac resynchronization therapy were the highest in stage D (**Table 1**).

Figure 5 shows the usage rates of medication in the CHART-2 patients. A total of 64.6% of patients were treated with renin–angiotensin system (RAS) inhibitors, and β-blockers were used in 40.4% of patients. The penetration rates of such standard medication for HF were the highest in stage C but decreased in stage D patients. Aldosterone inhibitors, digitalis, warfarin, and amiodarone were used most frequently in stage D patients.

Echocardiographic findings and LVEF are shown in **Figure 6**. As HF stage progressed, LV end-diastolic dimension was increased, LVEF was decreased, and the percentage of patients with low EF was increased. Patients with HFpEF comprised 69.1% and 51.1% of stage C and D subjects, respectively. B-type natriuretic peptide (BNP) level was also increased as HF stage progressed (**Table 1**).

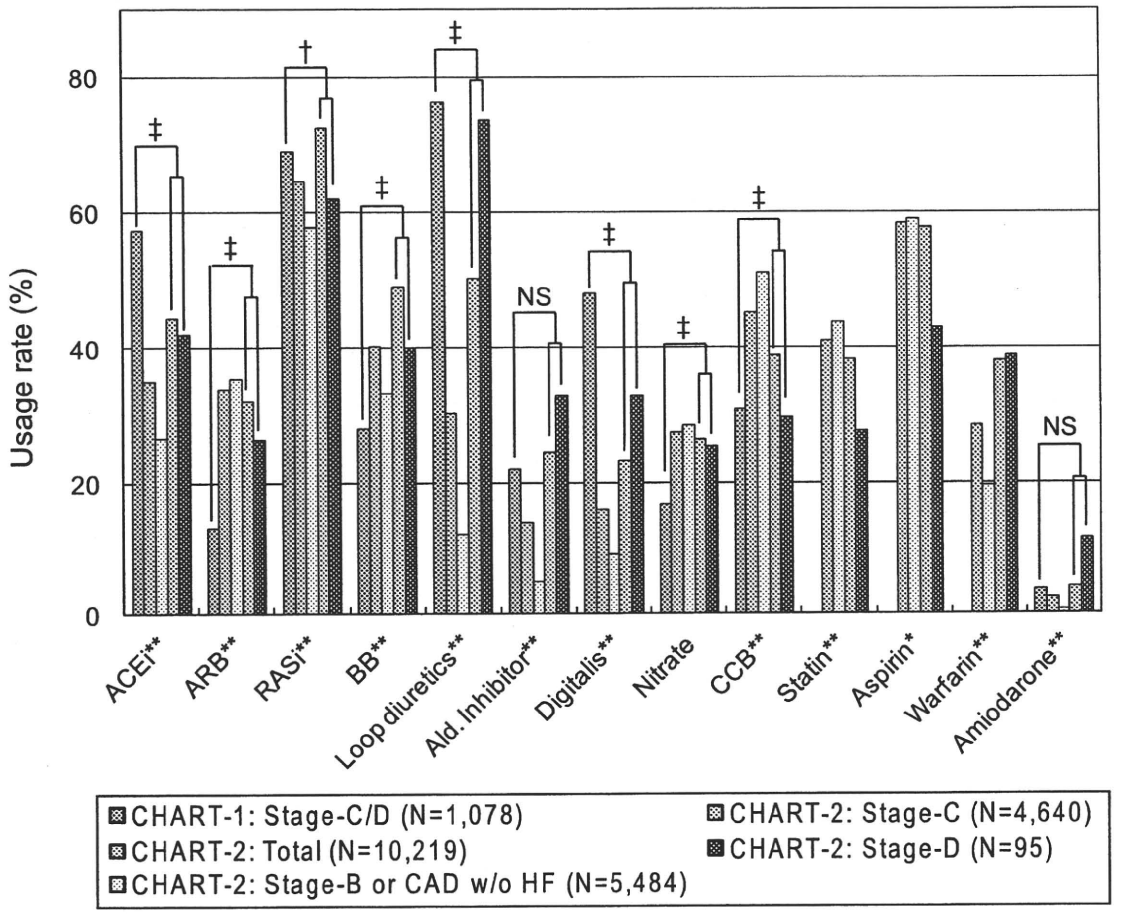

Figure 5. Medication of the CHART-1 and CHART-2 patients. ACEi, angiotensin-converting enzyme inhibitor; Ald., aldosterone; ARB, angiotensin II receptor blocker; BB, β-blocker; CAD, coronary artery disease; CCB, calcium channel blocker; RASi, renin–angiotensin system inhibitor; w/o, without. *P<0.05 and **P<0.001 between patients in stage B/CAD, stage C, and stage D in the CHART-2 Study. †P<0.05 and ‡P<0.001 between stage C/D patients in the CHART-1 Study and those in the CHART-2 Study.

Comparisons of Baseline Characteristics Between the CHART-1 Patients and the CHART-2 Patients or Those in Western Studies

The baseline characteristics of stage C/D patients enrolled in the previous CHART-1 Study[4,5] are given in Table 1 and Figures 3–6. Table 2 lists the comparisons of registration data in overt HF patients between CHART-1, CHART-2, and several observational Western cohort studies.

Mean age, blood pressure, and prevalence of CKD were similar between overt HF patients in the CHART-1 Study and those in the CHART-2 Study (Tables 1,2). As compared with the CHART-1 patients, however, those in the CHART-2 Study were characterized by a higher proportion having CAD as an etiology of HF (47.1%), the higher prevalence of histories of hypertension and diabetes (74.3% and 23.3%, respectively), more frequent HF admission history (53.0%), and a higher proportion having HFpEF (68.7%; Table 2; Figures 3–5). The usage rate of RAS inhibitors and β-blockers for overt HF patients in the CHART-1 and CHART-2 Studies increased from 69.1% to 72.3% and from 27.9% to 49.0%, respectively. In contrast, the usage rate of loop diuretics and digitalis decreased from 76.3% to 50.9% and from 48.1% to 23.5%, respectively (Figure 5).

Table 2 summarizes the baseline characteristics of overt HF patients in the CHART-1 Study, the CHART-2 Study, and Western observational cohort studies. Compared with Western patients, the CHART patients were characterized by less frequent ischemic etiology of HF, lower systolic blood pressure, less frequent diabetes, lower body mass index, and more frequent HFpEF. Usage rates of RAS inhibitors and β-blockers were similar between the CHART-2 patients and the Western HF patients except for the use of diuretics.

Characteristics of Patients in Stage B or Having CAD but Without HF

Patients in stage B or having CAD but without HF were characterized by younger age (67.6 years), a higher proportion of male patients (71.0%), less severe symptoms, and higher EF compared with patients in stages C or D (Table 1; Figure 6). The prevalence of cardiovascular risks such as hypertension, diabetes, and dyslipidemia, however, was similarly high (Figure 4), BNP was mildly elevated (Table 1), and the usage rate of standard HF treatment, such as RAS inhibitors and β-blockers, was too low in those patients (Figure 5).

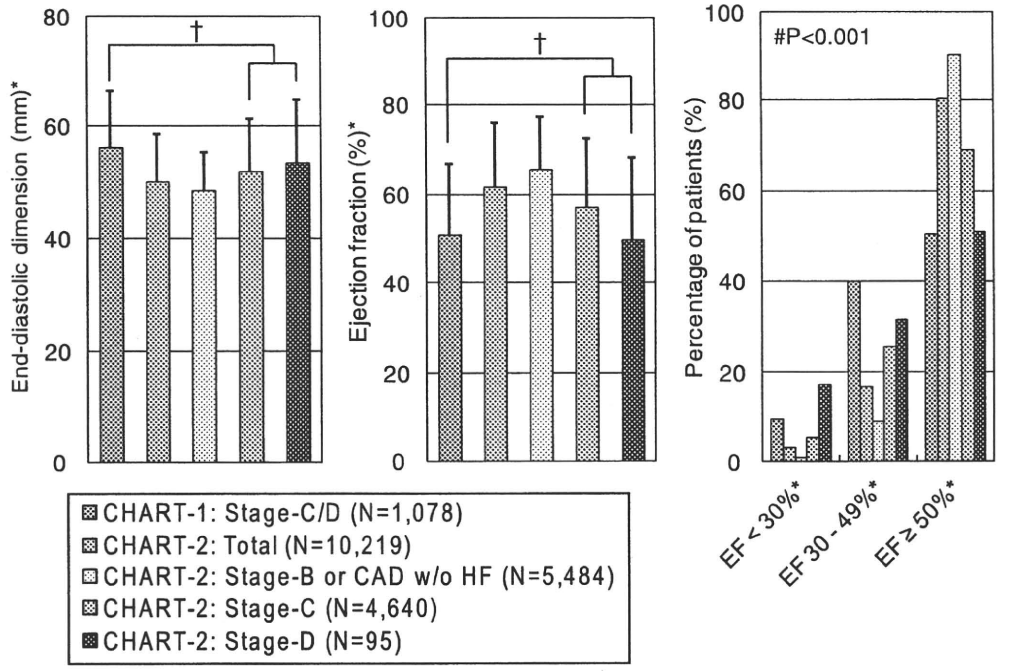

Figure 6. Left ventricular dimension and ejection fraction of the CHART-1 and CHART-2 patients. CAD, coronary artery disease; EF, ejection fraction. *P<0.001 between patients in stage B/CAD, stage C, and stage D in the CHART-2 Study. †P<0.001 between stage C/D patients in the CHART-1 Study and those in the CHART-2 Study. #P-value for the difference in distribution of ejection fraction between stage C/D patients in the CHART-1 Study and those in the CHART-2 Study.

Discussion

The clinical characteristics and prognosis of patients at high risk for disease progression due to development of AHFS have been poorly described, and thus epidemiological research involving such patients is extremely important in preventing the disease progression of HF and CVD. The CHART-2 Study is the first and the largest multicenter prospective cohort of consecutively enrolled patients at high risk for CVD progression due to AHFS in Japan. The Tohoku University head office and the CRC fulfilled their function to enroll patients in collaborating hospitals located in the Tohoku area, and the newly developed Web-based entry system also supported smooth entry of patient data.

Major Findings of the Present Analysis

Analysis of the registration data provides several new findings regarding patients with HF and those at risk of disease progression due to development of AHFS. First, when the CHART-2 patients were compared with the CHART-1 patients, a trend of increasing ischemic etiology and comorbidities of diabetes and hypertension was evident in Japanese patients with HF, whereas those risks had been more prominent in Western patients with HF (Table 2; Figures 3,4). Second, in the CHART-2 Study approximately 54% of patients were classified as being in stage B or having CAD without overt HF. In those patients, the plasma BNP concentration was mildly elevated and the cardiovascular risk profile was also similar to that of patients in stages C or D (Table 1; Figures 3–5). Third, the severity of prognostic risks including reduced EF, elevated BNP, comorbidity of CKD, and low hemoglobin level were exacerbated progressively as HF stage progressed in the CHART-2 patients (Table 1; Figures 4,6). Fourth, the prevalence of HFpEF patients was higher (68.7%) in the CHART-2 Study compared with the CHART-1 Study, demonstrating the trend of increasing prevalence of HFpEF (Figure 6).[12,13] Finally, the usage rates of standard medications in the CHART-2 patients were increased compared with the CHART-1 patients, but the usage was still too low, especially in the stage B patients (Figure 5).

Clear Trend of Increasing Prevalence of Ischemic HF in Japan

Several observational studies have previously demonstrated that the prevalence of CAD as an etiology in HF patients was 25–32% in Japan.[3,4,20,21] The prevalence of HF patients with ischemic etiology in the CHART-2 Study was dramatically increased compared with that in the CHART-1 Study, approaching the prevalence observed in Western subjects (Table 2, Figure 3). The prevalence of hypertension and diabetes, which are significant risks for developing CAD, similarly increased in the CHART-2 patients compared with the CHART-1 patients (Table 2, Figure 4). The report of the MIYAGI-AMI Registry Study showed the steady trend of increasing incidence of acute myocardial infarction in 30 years in Japan.[22] We speculate that the clear trend of increasing prevalence of CAD as an etiology of HF is due to the following reasons: (1) the number of CAD patients has been increasing due to accelerated westernization of lifestyle in Japanese people; and (2) the number of survivors after acute coronary event has dramatically increased due to the recent progress in treatment.

Table 2. Baseline Characteristics: CHART Patients vs. Previous Western HF Studies

	Framingham Study (1993)[16]	ADHERE (2005)[6]	EuroHeart Failure Survey II (2006)[8]	Owan et al (2006)[12]	Bhatia et al (2006)[13]	CHART-1 (Stage C/D, 2004)[4]	CHART-2 (Stage C/D, 2010)
No. patients	652	105,388	3,580	4,596	2,450	1,078	4,735
Age (years), mean±SD	70.0±10.8	72.4±14.0	69.9±12.5	73.0	73.1	68.7±13.4	68.9±12.3
Male (%)	51	48	61.3	55.5	52.4	64.5	68.4
Blood pressure (mmHg), mean±SD							
Systolic	150.9±27.6	144±32.6	NA	NA	150.0	126.3±19.1	126.3±19.2
Heart rate (/min), mean±SD	78.6±14.6	NA	NA	NA	NA	74.7±14.3	72.4±14.9
Comorbidity (%)							
Hypertension	74	73	NA	54.9	51.3	47.4	74.3
Diabetes	19	44	NA	33.7	36.3	19.5	23.3
Atrial fibrillation/flutter	NA	31	NA	34.5	26.6	42.3	31.0
Ventricular tachycardia	NA	8	NA	NA	NA	20.1	6.8
CKD	NA	30 (renal insufficiency)	NA	NA	20.1 (Cre <1.7 mg/dl)	49.5	47.3
History of HF admission	NA	NA	NA	NA	NA	27.2	53.0
Underlying disease (%)							
Ischemic	53.5	57	53.6	58.6	44.0	26.4	47.1
Hypertensive	23.6	NA	62.5	NA	NA	17.7	9.9
Valvular	16.0	NA	34.4	4.7	NA	23.8	23.8
BMI (kg/m²), mean±SD	27.2±5.3	NA	26.8	29.1	NA	23.0±3.7	23.8±3.9
LVEF (%), mean±SD	NA	34.4±16.1	38±15	44.1	39.0	50.9±16.0	56.9±15.5
≥50% (%)	NA	37†		47.2	35.9†	50.6	68.7
Medication (%)							
ACEI	NA	41	55.0	NA	NA	57.4	44.6
ARB	NA	12	9.3	NA	NA	13.1	31.8
β-blocker	NA	48	43.2	NA	NA	27.9	49.0
Loop diuretics	NA	70 (all diuretics)	71.2 (all diuretics)	NA	NA	76.3	50.9
Digitalis	NA	28	26.6	NA	NA	48.1	23.5
Nitrate	NA	26	NA	NA	NA	16.8	26.3
Amiodarone	NA	11 (all anti-arrhythmics)	12.9 (all anti-arrhythmics)	NA	NA	3.6	4.2

CKD, chronic kidney disease; LVEF, left ventricular ejection fraction; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker. Other abbreviations see in Table 1.
†Ejection fraction >40%.

Patients at High Risk for AHFS in the CHART-2 Study

Heart failure is classified according to the 4 stages of HF syndrome.[2] Stage A and stage B are pre-HF stages but appropriate identification and treatment are needed to prevent the progression to overt HF, which is equivalent to the development of de novo AHFS. In the present study, we enrolled patients without HF but with CAD, patients with structural heart disease but without HF (stage B), and patients with overt HF (stages C and D) in order to include patients at high risk for developing AHFS.

In Western HF patients, approximately 60–80% of patients hospitalized due to AHFS have a previous history of HF,[3,9,23] and the re-hospitalization rate following HF admission is 25% at 30 days after admission.[24] These findings suggest that patients in stages C or D are the most susceptible group to AHFS. Approximately one-third of AHFS cases are considered to be de novo AHF,[3,9,23] and the majority were related to CAD.[24,25] Other major comorbidities or cardiovascular risks in patients admitted with AHFS included hypertension, diabetes, arrhythmia and renal insufficiency.[3,9,23,25] In the present study, the stage B patients were characterized by a high number of cardiovascular risks along with some cardiac structural abnormalities, and 58.2% of those patients had CAD (Figures 3, 4). For these reasons, we also enrolled stage B patients and those with CAD but without HF, as patients at high risk for developing AHFS.

HF Stage Progression and Exacerbation of Cardiovascular Risk

Baseline characteristics of the CHART-2 patients showed the graded effects of HF stage on cardiovascular risk and comorbidity. As the HF stage progressed from stage B to stage D, mean age, number of female patients, heart rate, cardiothoracic ratio, LV dimension, and plasma BNP concentration increased significantly; whereas blood pressure, hemoglobin level, body mass index, waist circumference and EF decreased significantly (Table 1; Figures 3–6). In the present study the BNP level was mildly elevated in patients with CAD but without HF or in those in stage B, and was significantly increased with the decline of EF and exacerbation of HF stage (Table 1; Figure 6). It has also been reported that stage B patients had increased BNP level with heightened risk of mortality or cardiovascular events.[26,27] CKD is also an extensive public health problem and is more prevalent in patients with CVD or with CVD-related risk factors, such as hypertension, diabetes mellitus, dyslipidemia, and MetS.[28,29]

Furthermore, CKD is also a significant aggravating factor in those patients. As shown in Figure 4, the number of patients with CKD increased with the severity of HF stage. Anemia or low hemoglobin level is associated with poor prognosis in HF patients.[30] Hemoglobin level was decreased in the CHART-2 patients, reflecting the worsening in severity of HF and CKD in those patients (Table 1; Figure 4). MetS involves a cluster of important risk factors, including central obesity, elevated fasting plasma glucose, dyslipidemia, and high blood pressure and has become a leading health concern due to the strong link to CVD.[19] A recent meta-analysis of 87 studies reported that MetS is associated with a 2-fold increase in cardiovascular outcomes and a 1.5-fold increase in all-cause mortality.[31] Otherwise low body mass index has been consistently considered to be associated with the increased number of deaths in HF patients,[32] and the prognostic influence of MetS in those patients remains uncertain. The present study demonstrates that both body mass index and the prevalence of MetS in the CHART-2 patients were significantly decreased as HF stage progressed (Table 1; Figure 4).

Increasing Prevalence of HFpEF in the CHART-2 Study

Approximately half of the HF patients have normal or preserved EF, called HFpEF.[12,13,29] In the CHART-2 Study the prevalence of HFpEF was increased compared with the CHART-1 Study (68.7% vs. 50.6%; Table 2; Figure 6). Although the reason for the increasing prevalence of HFpEF remains unknown, we suggest the following: (1) the Japanese population is rapidly aging and the percentage of elderly HF patients has increased;[3] (2) the prevalence of hypertension has increased as a comorbidity of HF (Table 2); and (3) the recent progress in reperfusion therapy has contributed to preservation of EF after acute coronary events.[22]

Use of Standard Medication for CVD in the CHART-2 Patients

It has previously been reported that standard HF treatments were not used in patients who would have benefited from such medications.[33] The overall usage rates of RAS inhibitors or β-blockers in the CHART-2 patients were 64.6% and 40.4%, respectively (Figure 5). Although the penetration rate of such treatment was increased in overt HF patients in the CHART-2 Study compared with the CHART-1 Study (Table 2), it was still too low, especially in stage B patients (Figure 5). Further investigation is necessary to evaluate how such a low treatment rate of evidence-based medicine affects the prognosis of stage B patients.

Study Limitations

Several limitations in the design of the CHART-2 Study should be mentioned. First, the present study did not include data regarding physical inactivity, diet or nutrition, all of which are important modifiable risks for developing CVD. Second, all subjects in the CHART studies were Japanese people, which may limit extrapolation of the results to patients in Western countries. Third, the difference of the entry criteria in the CHART-1 and CHART-2 Studies might limit accurate comparison of enrolled patients in those 2 studies. Fourth, the primary design of the present study did not cover chronic lung disease, which has been recently recognized as one of the important cardiovascular risks.[34] In order to address this important issue, we started a retrospective survey on chronic obstructive pulmonary disease in the CHART-2 patients from April 2010.

Conclusions

The CHART-2 Study demonstrates the trend of increasing westernization of etiology, and the prevalence of hypertension and diabetes in HF patients in Japan. Although the number of HF patients is predicted to increase dramatically in the near future, the usage rate of standard medications in patients with CVD or HF is still too low, especially in stage B patients. Given the growing number of patients with CVD and HF in Japan, strategies preventing the development of CAD must be given top priority.

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Appendix 1

Study Organization of The CHART-2 Study

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Appendix 2

Subjects in stage B must meet at least one of the following criteria and must not have signs, symptoms, or history of hospitalization for heart failure.

(1) Enlarged left ventricular end-diastolic dimension (≥55 mm) measured on echocardiography.
(2) Impaired left ventricular ejection fraction (≤50%) measured on echocardiography.
(3) Thickened interventricular septum (>12 mm) and/or thickened left ventricular posterior wall (>12 mm) measured on echocardiography.
(4) Significant valvular stenosis/insufficiency.
(5) Significant myocardial abnormalities.
(6) Congenital abnormalities.
(7) Previous cardiac surgery.

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Ataxia Telangiectasia Mutated (ATM)-mediated DNA Damage Response in Oxidative Stress-induced Vascular Endothelial Cell Senescence*[S]

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Oxidative stress regulates dysfunction and senescence of vascular endothelial cells. The DNA damage response and its main signaling pathway involving ataxia telangiectasia mutated (ATM) have been implicated in playing a central role in mediating the actions of oxidative stress; however, the role of the ATM signaling pathway in vascular pathogenesis has largely remained unclear. Here, we identify ATM to regulate oxidative stress-induced endothelial cell dysfunction and premature senescence. Oxidative stress induced senescence in endothelial cells through activation/phosphorylation of ATM by way of an Akt/p53/p21-mediated pathway. These actions were abrogated in cells in which ATM was knocked down by RNA interference or inhibited by specific inhibitory compounds. Furthermore, the *in vivo* significance of this regulatory pathway was confirmed using ATM knock-out mice in which induction of senescent endothelial cells in the aorta in a diabetic mouse model of endothelial dysfunction and senescence was attenuated in contrast to pathological changes seen in wild-type mice. Collectively, our results show that ATM through an ATM/Akt/p53/p21-dependent signaling pathway mediates an instructive role in oxidative stress-induced endothelial dysfunction and premature senescence.

The DNA damage response is activated in response to stimuli ranging from oxidative stress, oncogenic stress to ionizing radiation to determine which cells remain viable after cytopathogenic insult. Recent reports suggest that the DNA damage response in addition to its classical role in regulating the cell cycle checkpoint in cancer to also play a major regulatory role in nononcogenic fields such as in aging/senescence (1–3). One field in which the DNA damage response has remained poorly addressed is the cardiovasculature.

Aging is known to be a major cardiovascular risk factor (4). Premature senescence in endothelial cells is induced by proatherogenic and proinflammatory factors such as hydrogen peroxide (H_2O_2), oxidized LDL or TNF-α by telomeric inactivation through an Akt-dependent mechanism (5, 6). Because regulation of aging/senescence of the vasculature, notably through the endothelial cell, contributes to mechanisms of arteriosclerosis and other age-related cardiovascular diseases (7), we questioned whether the DNA damage response might play a role in regulation of endothelial regulation of aging/senescence. For this, we focused on the role of ataxia telangiectasia mutated (ATM),[4] which is the central effector molecule in the DNA damage response pathway.

ATM belongs to the phosphoinositide 3-kinase (PI3-kinase)-related protein kinase (PIKK) family which has been identified as the product mutated or inactivated in ataxia telangiectasia (A-T) patients. The DNA damage response and its main signaling pathway involving ATM have been implicated in playing a central role in mediating the actions of oxidative stress (8–10). However, the role of the ATM signaling pathway in vascular pathogenesis has remained unclear. Further, pathogenic mechanisms of the vascular pathologies associated with mutated ATM (telangiectasia, premature coronary artery disease) remain obscure.

In the present study, we examined the effects of ATM-mediated oxidative stress-induced senescence in vascular pathologies through actions on endothelial cells. Our results show that ATM through an ATM/Akt/p53/p21-dependent signaling pathway mediates an instructive role in oxidative stress-induced endothelial dysfunction and premature senescence.

EXPERIMENTAL PROCEDURES

Cell Culture—Human umbilical vein endothelial cells (HUVECs) were purchased from Sanko Junyaku (Tokyo, Japan) and maintained with endothelial cell basal medium-2 containing EGM-2 supplement as purchased from Cambrex Bio Science (Rockland, MD) in humidified air with 5% CO_2 at 37 °C. All experiments were performed between passages 4 and 6.

Western Blot Analysis and Antibodies—HUVECs (3×10^5 cells/well) were treated with 100 μM H_2O_2 in the absence or presence of *N*-acetyl-L-cysteine (NAC) (Sigma-Aldrich), caffeine (Wako, Osaka, Japan), or KU-55933 (Calbiochem). Cells were washed with cold phosphate-buffered saline (PBS) and

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Experimental Procedures and Figs. 1–7.

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[4] The abbreviations used are: ATM, ataxia telangiectasia mutated; A-T, ataxia telangiectasia; BisTris, bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane; DSB, DNA double-strand break; HUVEC, human umbilical vein endothelial cell; NAC, *N*-acetyl-L-cysteine; SA-β-gal, senescence-associated β-galactosidase; STZ, streptozotocin.

lysed with 2× NuPAGE LDS sample buffer (Invitrogen) supplemented with 1 mM sodium orthovanadate (Sigma-Aldrich) and 1 mM sodium fluoride (Wako). Lysates were then boiled for 10 min and centrifuged for 2 min at 4 °C. Equal amounts of protein were separated by NuPAGE 3–8% Tris acetate mini gel electrophoresis or NuPAGE 10% BisTris gel (Invitrogen) and then transferred onto a polyvinylidine difluoride (PVDF) membrane (Invitrogen). Membranes were blocked with 5% skim milk in PBS at room temperature for 1 h and then subsequently probed with primary antibodies at a predetermined optimal concentration for 2–4 h at room temperature or overnight at 4 °C. After rinsing with TBS containing 0.1% Triton X-100 (TBS-T), membranes were incubated with appropriate horseradish peroxidase-conjugated anti-rabbit (Cell Signaling Technology) or anti-mouse IgG (GE Healthcare) for 1 h at room temperature. After three washes with TBS-T, immunoblots were detected using the ECL Plus Western blotting Detection System (GE Healthcare) and exposed to x-ray film (Fuji medical x-ray film, Tokyo, Japan). Anti-phospho-ATM (Ser^{1981}) antibody was purchased from Millipore; anti-ATM (2C1) and anti-p53 (DO-1) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA); and anti-phospho-p53 (Ser^{15}), anti-phospho-Akt (Ser^{473}), and anti-Akt antibodies were from Cell Signaling Technology. Anti-GAPDH antibody (Life Technologies) was used as a loading control. Purified mouse anti-p21 antibody was obtained from BD Pharmingen. See also supplemental Experimental Procedures.

Immunofluorescence Microscopy—Cells were grown on coverslips at a density of 1×10^5 per slide. After treatment with 100 μM H_2O_2 for 1 h, cells were fixed in 3% paraformaldehyde in PBS for 10 min. Chamber slides were washed three times with PBS and then permeabilized with 0.25% Triton X-100 in PBS for 10 min. After washing twice with PBS and blocking for 5 min with 0.1% gelatin, slides were incubated with primary antibody (1:100) in 0.1% gelatin in PBS for 1 h in a humidified chamber at 37 °C. Cells were blocked three times with 0.1% gelatin, and then samples were incubated with secondary antibody using Alexa Fluor 488 green or Alexa Fluor 635 red (Life Technologies) in 0.1% gelatin in PBS for 1 h in a humidified chamber at 37 °C. Antibodies used for immunofluorescence were ATM-Ser^{1981}, ATM, p53-Ser^{15}, p53, p21, and 53BP1 (Cell Signaling Technology). Nuclei were stained with Hoechst 33258 and mounted with DakoCytomation Fluorescent Mounting Medium (Dako Japan, Kyoto, Japan) and then visualized with a Carl Zeiss LSM510 confocal microscope (Carl Zeiss, Jena, Germany).

Senescence-induced by Oxidative Stress and Senescence-associated β-Galactosidase (SA-β-gal) Staining—Methods for inducing premature senescence by H_2O_2 (Wako) have been described previously (11). Briefly, HUVECs (3×10^5 cells/well) were grown in 30-mm collagen-coated dishes to 80% confluence. Cells were pretreated for 3 days with vehicle, NAC (0.1, 2, and 10 mM), caffeine (0.1, 2, and 5 mM), or KU-55933 (0.5, 5, and 10 μM). After washing three times with endothelial cell basal medium-2 and treating for 1 h with 100 μM H_2O_2, cells were trypsinized, reseeded at a density of 5×10^4 in 30-mm dishes, and cultured with endothelial cell basal medium-2 containing compound for 10 days. Cells were then washed in PBS and fixed for 5 min at room temperature in 2% formaldehyde/0.2% glutaraldehyde, washed, and then incubated at 37 °C for 12 h (without CO_2) with fresh SA-β-gal stain solution which contained 1 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal), 40 mM citric acid/sodium phosphate, pH 6.0, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM sodium chloride, and 2 mM magnesium chloride. Senescent cells were identified as blue-stained cells by phase contrast microscopy, and a total of 1,000 cells were counted in 20 random fields to determine the percentage of SA-β-gal-positive cells.

RNA Interference—Small interference RNA (siRNA) constructs were obtained as siGENOME SMARTpool reagent from Dharmacon. See also supplemental Experimental Procedures. A siRNA pool specific for ATM (siGENOME SMARTpool Human ATM, M-003201020005) and control siRNA (siGENOME nontargeting siRNA pool, D-0012061320) were used. HUVECs (3×10^5 cells/well) were grown to 80% confluence in 6-well culture dishes. Transient transfections of 10, 50, and 100 pmol of ATM siRNA or nontarget siRNA were performed by a liposome-mediated method using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen). The indicated amounts of siRNA and 5 μl of Lipofectamine 2000 were, respectively, diluted in 0.25 ml of Opti-MEM without serum. After incubation for 5 min, diluted siRNA and Lipofectamine 2000 were combined, and the mixture was incubated for 20 min at room temperature to allow the DNA·Lipofectamine 2000 complexes to form. 0.5 ml of complex was added to each well. After 5 h of transfection, the medium with complexes was removed, and endothelial cell basal medium-2 supplemented with EGM-2 was added. At 72 h following transient transfection, total RNA was extracted and submitted to reverse transcription-PCR (RT-PCR) experiments using oligonucleotide primers specific to ATM and 18 S rRNA. In ATM knock-down cells, the levels of total ATM, phospho-p53 (Ser^{15}), phospho-Akt (Ser^{473}), total Akt, or GAPDH proteins were analyzed by Western blotting, or SA-β-gal activity was measured.

RNA Extraction and Quantitative RT-PCR—Total RNA from HUVECs (3×10^5 cells/well) was extracted by the RNeasy Mini kit (Qiagen) according to the manufacturer's instructions. RNA concentration and purity were determined on a spectrophotometer (Ultrospec 3000; GE Healthcare) by calculating the ratio of optical density at 260 nm to 280 nm. One microgram of total RNA sample was used to generate first-strand complementary DNA by using powerscript reverse transcriptase (Clontech) according to the manufacturer's recommended procedures. PCR was then performed for ATM from the same complementary DNA samples using HotStarTaq (Qiagen), 10× PCR buffer, and 2.5 mM dNTP mix. The forward primer 5′-GATGTTGTTGTCCCTACTATGG-3′ and the reverse primer 5′-GCTACACTGCGCGTATAAGCC-3′ corresponded to human ATM. 18 S rRNA (Life Technologies) was used as an internal control. Amplification was initiated by 15 min of denaturation at 95 °C for 1 cycle followed by 30 cycles at 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 40 s. After the last cycle of amplification, samples were incubated at 72 °C for 10 min in a GeneAmp™ PCR system (Applied Biosystems). PCR products, a 589-bp ATM fragment and a 350-bp 18 S fragment, were then visualized by UV

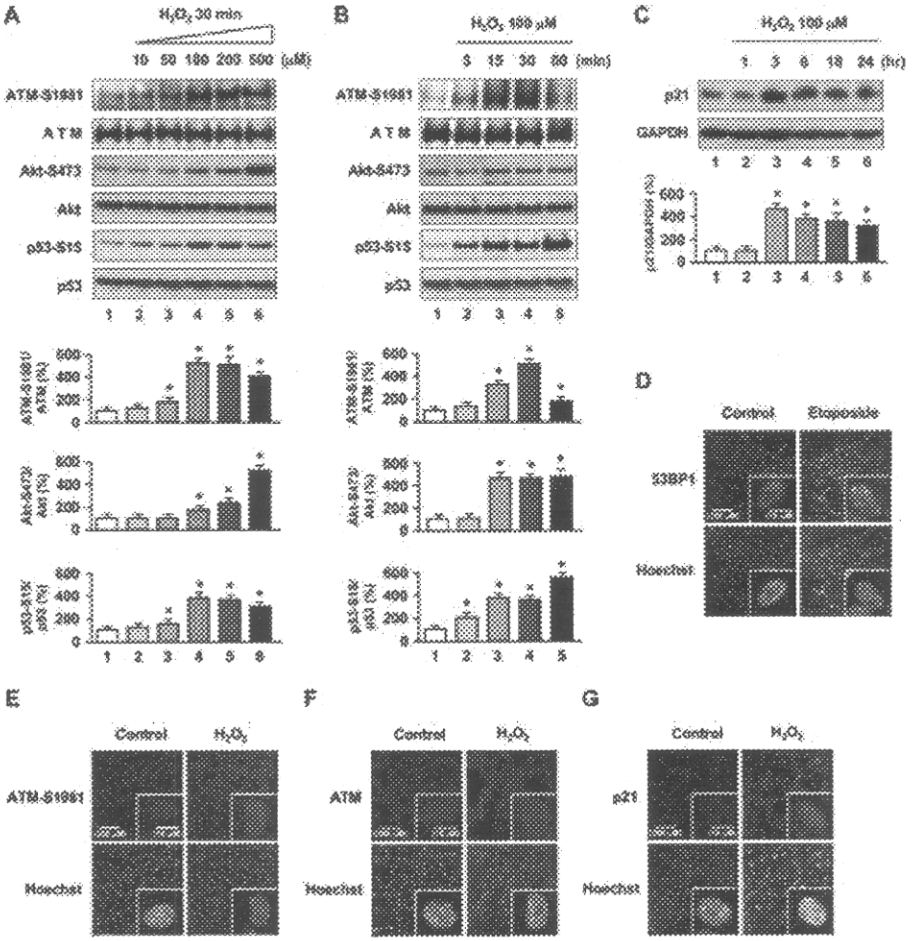

FIGURE 1. **Oxidative stress induces ATM-S1981, Akt-S473, p53-S15 phosphorylation and up-regulation of p21 expression in HUVECs.** *A*, cells were incubated with H_2O_2 for 30 min at the indicated concentrations. *B* and *C*, HUVECs were incubated in 100 μM H_2O_2 for the indicated times. Cells were lysed and subjected to Western blot analyses with the indicated antibodies. GAPDH was used as loading control. Values are mean ± S.E. (*error bars*) (n = 3). *, $p < 0.05$ *versus* cells incubated without H_2O_2 treatment. Representative blots are shown in the *upper panels* whereas corresponding quantitation is shown in the *lower panels*. *D–F*, immunofluorescence for 53BP1, phosphorylated ATM-S1981, and total ATM is shown. Cells were exposed to 10 μM etoposide or 100 μM H_2O_2 for 1 h and then immunostained for 53BP1 (*D*), ATM-S1981 (*E*) and total ATM (*F*). Cells incubated without etoposide or H_2O_2 were used as controls. *G*, cells were exposed to 100 μM H_2O_2 for 3 h and then immunostained for p21. Hoechst 33258 was used as nuclear stain (*blue*). Original magnification, ×200 and ×630. *Scale bars*, 200 μm and 10 μm, respectively. Treatment with etoposide and immunofluorescence for 53BP1 (labeled by green fluorescence) was used for a positive control. Expression of phosphorylated ATM-S1981) and p21 labeled by red fluorescence in HUVECs and foci formation was significantly increased after H_2O_2 treatment. Additional views of the photographs are shown in supplemental Fig. 1.

illumination after electrophoresis by 2% agarose gels containing 0.5 μg/ml ethidium bromide.

Animal Experiments—ATM knock-out mice (129S6/SvEvTac-*Atm*^{tm1Awb}/J) were obtained from Jackson Laboratories. All generations were from matings of heterozygous parents. The following sequence, as recommended by Jackson Laboratories, was used for genotyping: oIMR640, 5′-GCTGCCATACTTGATCAATG-3′; oIMR641, 5′-TCCGAATTTGCAGGAGTG-3′; oIMR0013, 5′-CTTGGGTGGAGAGGCTATTC-3; and oIMR0014, 5′-AGGTGAGATGACAGGAGATC-3′. Age-matched 10-week-old SPF male ATM wild-type ($^{+/+}$), heterozygote ($^{+/-}$), and homozygote ($^{-/-}$) mice (n = 6, respectively, weighing ~15–25 g) were used. Hyperglycemia was induced by a single intraperitoneal injection of streptozotocin (STZ) (180 mg/kg; Sigma-Aldrich). Tail blood glucose was assayed 3 days after injection using glucose test strips (Roche Applied Science). All diabetic animals had blood glucose values >300 mg/dl. Mice were housed under constant temperature (23 ± 1 °C) with a 12-h light and 12-h dark cycle for 10 days with free access to water and chow and were killed by cervical dislocation. The aorta was removed after systemic perfusion with PBS for histological examination. The aorta was fixed for 30 min at room temperature in 2% formaldehyde/0.2% glutaraldehyde, washed, incubated at 37 °C for 24 h (without CO_2) with fresh SA-β-gal stain solution, and then embedded in OTC compound before freezing in liquid nitrogen. The samples were stored at −80 °C until sample slides were prepared. The proportion of SA-β-gal-positive cells were analyzed by Scion Image software. Serial cross-sections (10 μm) were obtained from each sample and stained with kernechtrot staining solution (Muto, Tokyo, Japan) or prepared for immunohistochemistry. All experimental protocols complied with the guidelines for animal experiments of the University of Tokyo.

Tissue Protein Extraction—Thoracic aorta of ATM knock-out mice (ATM$^{+/+}$, ATM$^{+/-}$, ATM$^{-/-}$ mice) were dissected out of their thoracic aortas and snap frozen in liquid nitrogen. After thawing on ice, the thoracic aortas were homogenized mechanically at 25 Hz for 25 s five times on ice in 150 μl of RIPA buffer (0.1% SDS, 0.5% Nonidet P-40, 0.1% sodium deoxycholate, 150 mM NaCl, 50 mM Tris-HCl, pH 7.9, and 1× EDTA-free complete proteinase inhibitor (Roche Applied Science)). Samples were lysed gently on ice for 30–60 min, and cellular debris was removed by centrifugation. Protein was then quantified using the BCA protein assay kit (Thermo). A 30-μg aliquot of total protein was analyzed for ATM protein by Western blot analysis. Anti-GAPDH antibody was used as a loading control.

Immunohistochemistry—Immunostaining was performed using the Envision kit (Dako Japan). Frozen sections, 10 μm thick, were fixed in acetone at 4 °C, washed in TBS, and then blocked by 0.03% hydrogen peroxidase in methanol. After blocking nonspecific antibody-binding sites, the sections were incubated for 1 h with antibodies against von Willebrand factor (1:1,000; Abcam), p21 and p16 (Santa Cruz Biotechnology) as the primary antibody and then for 1 h with the peroxidase-