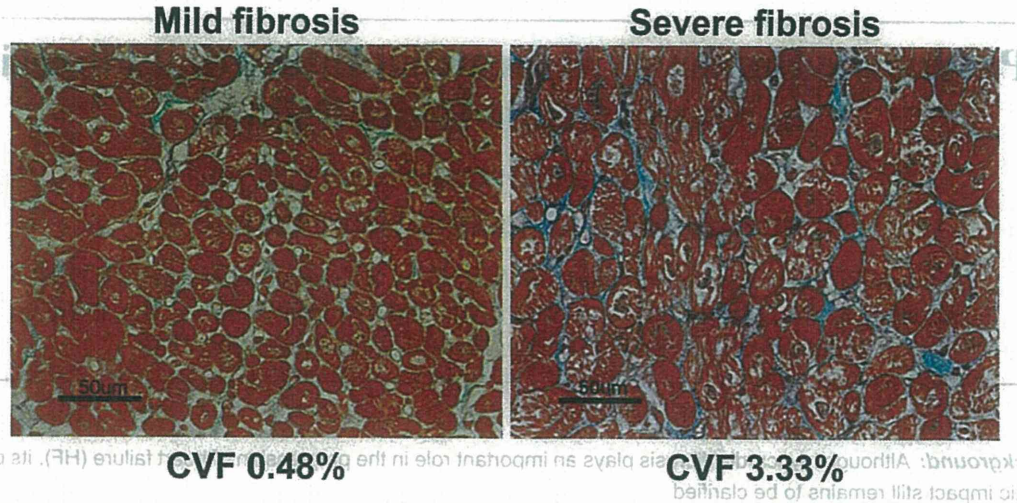

Figure 1. Representative histology of the (**Left**) mild and (**Right**) severe fibrosis groups. Myocardial fibrosis, stained in blue by Elastica–Masson staining. CVF, collagen volume fraction. Scale bar, 50 μm.

Methods

The ethics committees of Tohoku University Hospital approved the study protocol and all patients provided written informed consent.

Subjects

We examined 172 consecutive patients with chronic HF enrolled in the Tohoku University Hospital database, and who underwent cardiac catheterization and endomyocardial biopsy to determine the etiology of HF between January 2001 and September 2008. We performed endomyocardial biopsy in all HF patients with suspected cardiomyopathy but we did not perform the procedure in those who had apparent ischemic or valvular heart disease documented on echocardiography and/or cardiac catheterization.

For each patient, we collected clinical, hemodynamic, biochemistry and prognostic data and analyzed endomyocardial biopsy samples.

Definition of HF

In the present study, we included patients in stage B, C and D, according to the chronic HF ACC/AHA 2005 guidelines. According to the ESC 2007 HF guideline, we also divided them into 2 groups: HF with preserved ejection fraction (HFPEF; LVEF ≥50%, n=81) and HF with reduced LVEF (HFREF; LVEF <50%, n=91).

Data Collection

Baseline demographic data, hemodynamic data obtained via catheterization, stage of HF, medications and comorbidities (hypertension, diabetes mellitus, hyperlipidemia, and atrial fibrillation) were obtained based on medical records. The hemodynamic parameters measured via cardiac catheterization included LVEF, LV end-diastolic volume index (LVEDVI), mean aortic pressure, LV end-diastolic pressure (LVEDP), mean pulmonary artery pressure, pulmonary capillary wedge pressure (PCWP) and cardiac index. Before cardiac catheterization, we measured serum levels of hemoglobin, brain natriuretic peptide (BNP), creatinine and high-sensitivity C-reactive protein and estimated creatinine clearance using the Cockroft-Gault formula.

The primary endpoints included all-cause death, and the secondary combined endpoints included cardiovascular death, sudden death and admission for worsening of HF. Follow-up data were obtained from the database.

Quantitative Morphometry of Biopsy Samples

Trans-venous endomyocardial biopsy samples were obtained from the interventricular septum using 6-Fr Biotom (Cordis, Bridgewater, NJ, USA). There were no major complications related to the procedures during the study period. The tissues were immediately fixed in 10% buffered formalin and embedded in paraffin. Tissue sections were stained with hematoxylin–eosin and Elastica–Masson. Images of these sections were acquired with a projection microscope (×400; **Figure 1**). Subsequent image analysis was performed using Macscope 2.5 (Mitani, Fukui, Japan) to determine cardiomyocyte diameter and extent of myocardial interstitial fibrosis, which was expressed as CVF (%). CVF was calculated as the sum of all connective tissue areas divided by the sum of all connective tissue and muscle areas averaged over 2–5 representative fields of the section (mean, 3.6±0.9 fields), where there was no endocardium or blood vessel.[16,17] Myocardial diameter was determined at the nucleus level in 8–15 representative cardiomyocytes (mean, 12.0±2.5 fields) per section, where we also counted the number of inflammatory mononuclear cells in the same fields (mean, 6.0±1.8). This histological evaluation was performed by a well-trained cardiologist without knowledge of which patient provided the tissue sections.

We divided both the HFPEF and HFREF groups into 2 groups using median CVF (HFPEF and HFREF, 1.36% and 1.34%, respectively). We defined mild and severe fibrosis as CVF smaller and greater than the median, respectively (**Figure 1**).

Statistical Analysis

Continuous variables are expressed as mean±SD. Comparisons between 2 groups were conducted using unpaired t-test for continuous variables and chi-squared test for categorical

Table 1. Baseline Subject Characteristics

	HFPEF (n=81)	HFREF (n=91)	P value
Age (years)	54.3±14.1	55.9±12.8	0.429
BMI (kg/m²)	23.4±4.5	23.9±4.2	0.408
Male	54 (67)	66 (73)	0.404
Hypertension	34 (42)	39 (43)	0.962
Diabetes mellitus	9 (11)	13 (14)	0.533
Dyslipidemia	21 (26)	21 (23)	0.631
Sinus rhythm	69 (85)	65 (71)	0.028*
Medication			
ACEI	36 (44)	57 (63)	0.017*
ARB	15 (19)	35 (38)	0.004*
β-blocker	39 (48)	66 (73)	0.001*
Diuretics	13 (16)	41 (45)	<0.001*
Spironolactone	10 (12)	31 (34)	0.001*
Warfarin	12 (15)	46 (51)	<0.001*
Digitalis	7 (9)	28 (31)	<0.001*
CCB	26 (32)	11 (12)	0.001*
Antiplatelet	13 (16)	27 (30)	0.033*
Statin	7 (9)	17 (19)	0.054
Amiodarone	5 (6)	8 (9)	0.514
Stage of heart failure			<0.001*
B	40 (49)	15 (16)	
C	39 (48)	68 (75)	
D	2 (2)	8 (9)	
Laboratory data			
Hemoglobin (g/dl)	13.8±2.0	14.1±1.8	0.277
hsCRP (mg/dl)	0.21±0.46	0.33±0.93	0.294
BNP (pg/ml)	248±342	367±491	0.089
LDL (mg/dl)	110±41	121±39	0.103
HDL (mg/dl)	54.9±24.0	45.1±12.8	0.001*
TG (mg/dl)	131±98	137±75	0.669
Glucose (mg/dl)	106±37	106±21	0.934
CCr (ml/min)	90.1±25.1	88.2±35.4	0.694
Hemodynamic data			
LVEDVI (ml/m²)	75.7±20.4	114.5±35.1	<0.001*
EF (%)	67.8±11.3	35.6±11.0	<0.001*
mAoP (mmHg)	96±15	90±17	0.023*
LVEDP (mmHg)	14±6	13±7	0.420
mPAP (mmHg)	16.7±4.8	19.9±7.7	0.002*
PCWP (mmHg)	9.5±4.2	11.2±6.5	0.050
Cardiac index (L·min⁻¹·m⁻²)	2.9±0.7	2.6±0.7	0.021*
Morphometric data			
CVF (%)	1.83±1.54	2.07±2.35	0.440
MyD (μm)	19.2±3.2	19.7±2.8	0.362
Inflammatory cell (/field)	4.9±4.9	7.0±6.0	0.015*
All-cause death	0 (0)	9 (10)	0.004*
Cardiac events	4 (5)	15 (16)	0.016*
Cardiac or sudden death	0 (0)	4 (4)	
Admission for HF	4 (5)	11 (12)	

Data given as mean±SD or n (%). *P<0.05, HFPEF vs. HFREF.
HFPEF, heart failure patients with preserved left ventricular ejection fraction; HFREF, heart failure patients with reduced left ventricular ejection fraction; BMI, body mass index; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium cannel blocker; hsCRP, high-sensitivity C-reactive protein; BNP, brain natriuretic peptide; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CCr, creatinine clearance; LVEDVI, left ventricular end-diastolic volume index; EF, ejection fraction; mAoP, mean aortic pressure; LVEDP, left ventricular end-diastolic pressure; mPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; CVF, collagen volume fraction; MyD, cardiomyocyte diameter; HF, heart failure.

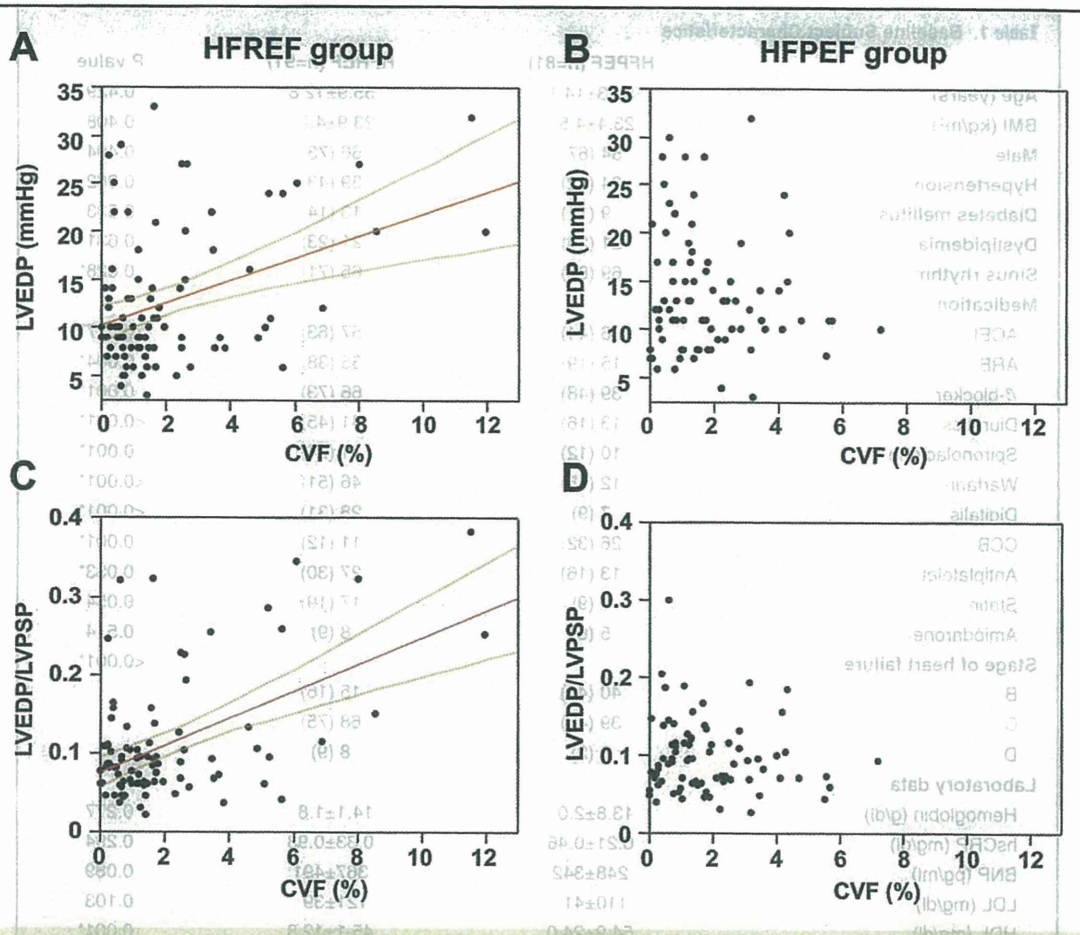

Figure 2. Correlation between left ventricular end-diastolic pressure (LVEDP) and collagen volume fraction (CVF). (**A**) A significant correlation was noted between CVF and LVEDP for patients with heart failure (HF) with reduced left ventricular ejection fraction (HFREF; r=0.387, P<0.001) but not for those with (**B**) HF with preserved left ventricular ejection fraction (HFPEF; r=–0.092, P=0.664). (**C**) LVEDP/left ventricular peak systolic pressure (LVEDP/LVPSP) ratio and CVF were also significantly correlated in the HFREF group (r=0.515, P<0.001), but not in the (**D**) HFPEF group (r=–0.097, P=0.393).

variables. For echocardiographic comparison before and after medical treatment, paired t-test was used. Five-year survival free from all-cause death and that from cardiac events was estimated using the Kaplan-Meier method. We used Cox proportional hazards model to adjust covariates. After comparison of covariates between the mild and severe fibrosis groups, the covariates with P<0.05 were used in the final multivariate models. Furthermore, we evaluated the prognostic value of CVF as a continuous variable. We used the variables with P<0.05 on univariate analysis in the final multivariate models, in which age, cardiac index, LV filling pressure, and stage of HF were controlled for, and we chose the parameters for final models using the step-up method. In these analyses, we used PCWP as a parameter of LV filling pressure, because LVEDP data were lacking in 3 cases. Furthermore, as previously reported,[18] we tested the proportionality assumptions of each parameter of the final models, with P<0.05 indicating non-proportionality. All statistical analysis was performed using JMP 7.0.2 (SAS Institute, Cary, NC, USA) and R 2.8.1(www.r-project.org/). All P-values were 2-sided, and P<0.05 was considered to be statistically significant.

Results

HFPEF Group vs. HFREF Group

All patients were successfully followed up in the present study. Mean follow-up period in the HFPEF and the HFREF groups was 41±33 months and 41±26 months, respectively. The HFREF group was characterized by more advanced stage of HF (Table 1). There were more all-cause deaths and cardiac events in the HFREF group than in the HFPEF group (Table 1). Five-year prognosis was significantly lower in the HFREF group than in the HFPEF group, in terms of survival from all-cause death (P=0.006) and survival from cardiac events (P=0.034). After the adjustment of HF stage of HF, however, there was no significant difference in cardiac events between the 2 groups.

The prevalence of the use of medications for HF at cardiac catheterization, including angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), β-blockers, diuretics, spironolactone and digitalis, was significantly higher in the HFREF than in the HFPEF group (Table 1). In contrast, the use of calcium cannel blockers (CCB) was more common in the HFPEF group (Table 1). The HFREF group had significantly larger LV volume, lower LVEF and lower cardiac index compared with the HFPEF group

Table 2. Subject Characteristics vs. Level of Fibrosis

	HFPEF			HFREF		
	Mild fibrosis (n=40)	Severe fibrosis (n=41)	P value	Mild fibrosis (n=46)	Severe fibrosis (n=45)	P value
Age (years)	57±11	52±16	0.082	58±12	54±14	0.21
BMI (kg/m²)	23±5	23±4	0.96	24±4	24±4	0.372
Male	29 (73)	25 (63)	0.238	34 (74)	32 (70)	0.949
Hypertension	13 (33)	21 (53)	0.164	21 (46)	18 (39)	0.739
Diabetes mellitus	5 (13)	4 (10)	0.906	5 (11)	8 (17)	0.521
Dyslipidemia	10 (25)	11 (28)	0.894	12 (26)	9 (20)	0.66
Sinus rhythm	31 (78)	38 (93)	0.281	31 (67)	34 (76)	0.389
Medication						
ACEI	17 (43)	19 (48)	0.941	28 (61)	29 (63)	0.892
ARB	7 (18)	8 (20)	0.874	18 (39)	17 (37)	0.934
β-blocker	19 (48)	20 (50)	0.902	32 (70)	34 (74)	0.685
Diuretics	7 (18)	6 (15)	0.884	17 (37)	24 (52)	0.174
Spironolactone	7 (18)	3 (8)	0.255	16 (35)	15 (33)	0.94
Warfarin	5 (13)	7 (18)	0.862	20 (43)	26 (57)	0.248
Digitalis	5 (13)	2 (5)	0.371	17 (37)	11 (24)	0.287
CCB	14 (35)	12 (30)	0.64	6 (13)	5 (11)	0.969
Antiplatelet	6 (15)	7 (18)	0.884	14 (30)	13 (28)	0.946
Statin	2 (5)	5 (13)	0.491	8 (17)	9 (20)	0.96
Amiodarone	5 (13)	0 (0)	0.053	3 (7)	5 (11)	0.687
Stage of heart failure			0.236			0.577
B	23 (58)	17 (43)		9 (20)	6 (13)	
C	16 (40)	23 (58)		34 (74)	34 (74)	
D	1 (3)	1 (3)		3 (7)	5 (11)	
Laboratory data						
Hemoglobin (g/dl)	14±2	14±2	0.357	14±2	14±2	0.674
hsCRP (mg/dl)	0.16±0.29	0.26±0.58	0.317	0.35±1.12	0.32±0.69	0.888
BNP (pg/ml)	255±377	243±314	0.892	245±347	494±584	0.019*
LDL (mg/dl)	108±38	113±44	0.635	116±38	125±40	0.239
HDL (mg/dl)	52±21	58±26	0.269	45±14	45±11	0.952
TG (mg/dl)	125±75	137±116	0.603	145±86	129±62	0.305
Glucose mg/dl)	112±43	100±29	0.134	104±19	109±23	0.315
CCr (ml/min)	90±25	90±26	0.898	89±30	87±40	0.804
Hemodynamic data						
LVEDVI (ml/m²)	76±18	75±22	0.874	107±28	122±40	0.040*
EF (%)	66±11	69±11	0.275	38±11	33±11	0.053
mAoP (mmHg)	97±16	96±15	0.775	92±15	88±19	0.189
LVEDP (mmHg)	14±7	13±6	0.236	11±6	14±8	0.06
mPAP (mmHg)	17±4	17±5	0.736	19±7	21±9	0.136
PCWP (mmHg)	10±4	9±4	0.926	10±5	12±8	0.161
Cardiac index ($L \cdot min^{-1} \cdot m^{-2}$)	2.9±0.7	2.9±0.7	0.932	2.7±0.7	2.6±0.6	0.367
Morphometric data						
CVF (%)	0.64±0.41	2.93±1.38	<0.001*	0.61±0.4	3.56±2.58	<0.001*
MyD (μm)	19±1.9	20±4.1	0.287	19±2	20±3	0.055
Inflammatory cell (/field)	4.7±4.6	5.1±5.2	0.696	8±6	6±6	0.116
All-cause death	0 (0)	0 (0)	–	1 (2)	8 (18)	0.013*
Cardiac events	3 (8)	1 (2)	0.293	3 (7)	12 (27)	0.001*
Cardiac or sudden death	0 (0)	0 (0)		1 (2)	3 (7)	
Admission for HF	3 (8)	1 (2)		2 (4)	9 (20)	

Data given as mean±SD or n (%). *P<0.05, mild fibrosis vs. severe fibrosis.
Abbreviations see in Table 1.

(Table 1). Although LVEDP and CVF were comparable between the 2 groups (Table 1), CVF was significantly correlated with LVEDP, and also with LV peak systolic pressure (LVPSP) after adjustment in the HFREF group (Figures 2A,C), but not in the HFPEF group (Figures 2B,C).

Morphometric Variables as Prognostic Indicators

When comparing the mild and the severe fibrosis groups, a statistically significant difference was noted in terms of LVEDVI and BNP in the HFREF group (Table 2), but not in the HFPEF group (Table 2).

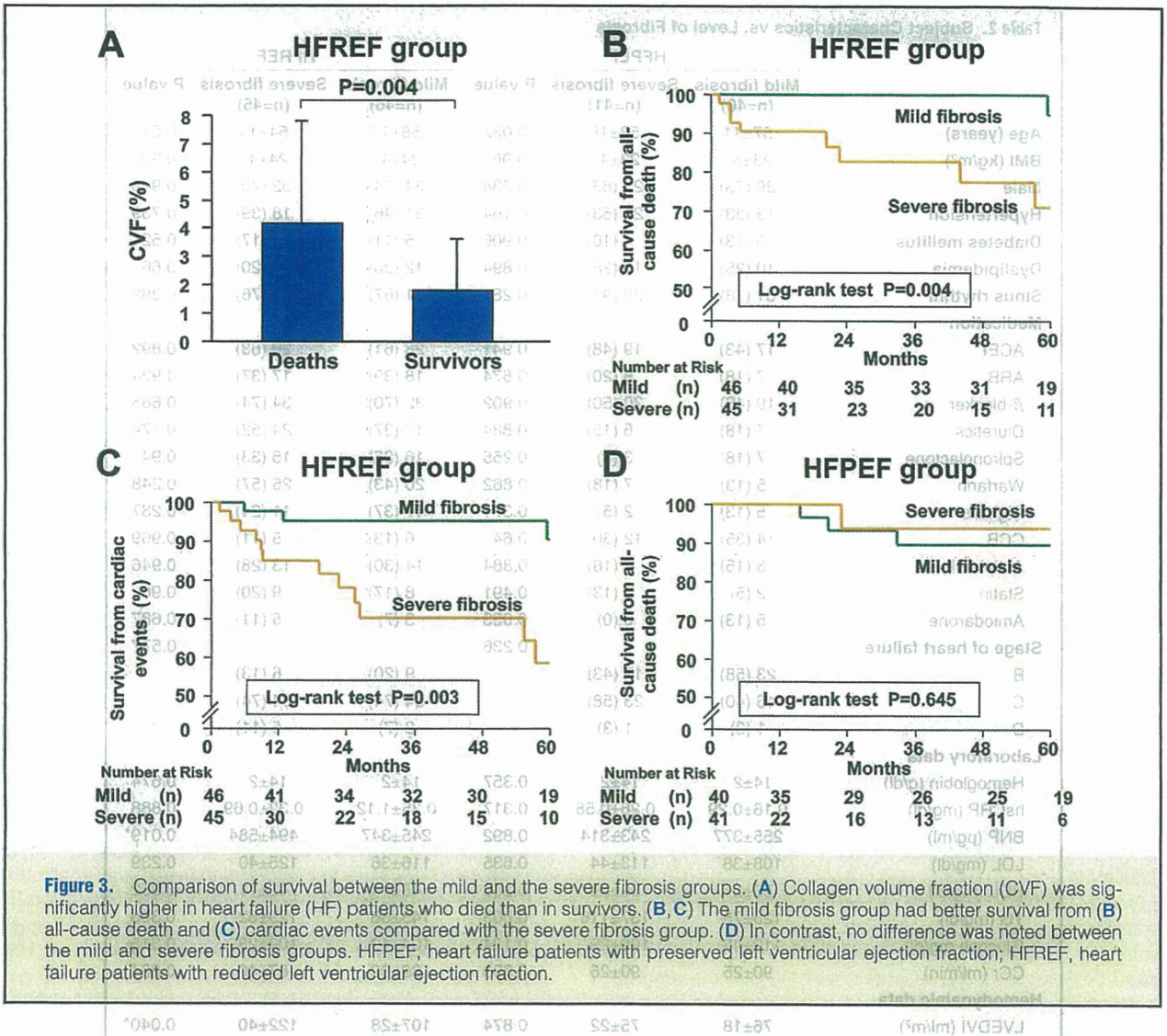

Figure 3. Comparison of survival between the mild and the severe fibrosis groups. (**A**) Collagen volume fraction (CVF) was significantly higher in heart failure (HF) patients who died than in survivors. (**B**, **C**) The mild fibrosis group had better survival from (**B**) all-cause death and (**C**) cardiac events compared with the severe fibrosis group. (**D**) In contrast, no difference was noted between the mild and severe fibrosis groups. HFPEF, heart failure patients with preserved left ventricular ejection fraction; HFREF, heart failure patients with reduced left ventricular ejection fraction.

In the HFREF group, CVF was significantly higher in HF patients who died than in survivors (Figure 3A). Indeed, there were more all-cause deaths and cardiac events in the severe fibrosis group than in the mild fibrosis group (Table 2). Five-year survival from all-cause death was significantly lower in the severe fibrosis group than in the mild fibrosis group (P=0.004; Figure 3B), and was so even after adjustment with the covariate (severe fibrosis vs. mild fibrosis; hazard ratio [HR], 13.5; 95% confidence interval [CI]: 2.01–307, P=0.006). Similarly, survival after cardiac events was significantly lower in the severe fibrosis group than in the mild fibrosis group in the HFREF subjects (P=0.003; Figure 3C), and was so even after adjustment with the covariate (severe fibrosis vs. mild fibrosis; HR, 6.20; 95%CI: 1.52–25.4, P=0.011). In contrast, in the HFPEF group, there was no significant difference in the cardiac events (Table 1) or survival rate (Figure 3D) between the mild and severe fibrosis groups. In the HFREF group, multivariate analysis showed that a 1% elevation of CVF increased the risk of all-cause death and that of cardiac events by 1.50-fold (95%CI: 1.18–1.95, P=0.002) and 1.28-fold (95%CI: 1.07–1.50, P=0.008), respectively (Figure 4). Furthermore, other histological parameters (eg, cardiomyocyte hypertrophy) were not significant predictors in the present study.

Discussion

The novel findings of the present study are as follows: (1) CVF was similar between the HFPEF and HFREF groups; (2) CVF was an independent predictor of all-cause death and cardiac events in the HFREF group but not in the HFPEF group; and (3) CVF was significantly correlated with LVEDP in the HFREF group but not in the HFPEF group. To the best of our knowledge, this is the first report to demonstrate the prognostic impact of CVF in non-ischemic HF patients with systolic dysfunction.

HFPEF Group vs. HFREF Group

Several studies have shown that the prognosis is comparable between patients with HFPEF and those with HFREF.[19–21] In the present study, the patients with HFPEF had a significantly better prognosis than those with HFREF, but after adjustment for stage of HF, the survival became similar between the 2 groups. In the present study, the 5-year survival rate from all-cause death was better than in the previous study,[22] probably because we followed up the patients monthly to control sodium intake and blood pressure. It has been reported that intensive medical treatment for HF patients with close fol-

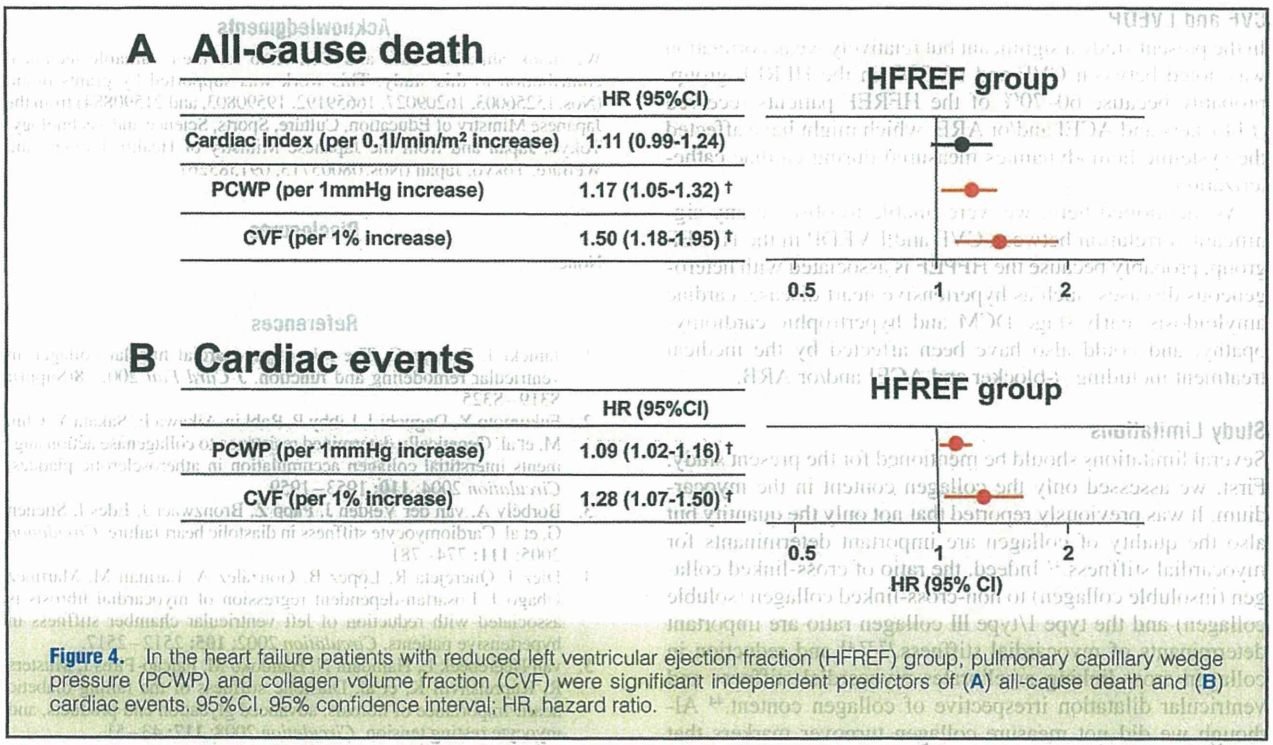

Figure 4. In the heart failure patients with reduced left ventricular ejection fraction (HFREF) group, pulmonary capillary wedge pressure (PCWP) and collagen volume fraction (CVF) were significant independent predictors of (A) all-cause death and (B) cardiac events. 95%CI, 95% confidence interval; HR, hazard ratio.

low-up can reduce re-admission for HF and cardiac deaths,[23] suggesting that the regular follow-up in the present study was effective to improve the prognosis of the HF patients.

Morphometric Variables and Cardiac Function as Prognostic Indicators

Myocardial fibrillar collagen, the main component of ECM, is a major contributor to myocardial stiffness.[3] In the present study, CVF in the HFPEF and the HFREF groups was 1.83% and 2.07%, respectively, consistent with the previous report.[24]

Recently, degradation of interstitial collagen has been reported in patients with mild to moderate dilated cardiomyopathy (DCM).[25,26] In contrast, marked accumulation of myocardial interstitial fibrosis has also been reported in patients with end-stage HFREF (eg, explanted heart).[27] The present study also demonstrated that CVF was significantly higher in HF patients who died than in survivors and that CVF and LVEDP were significantly correlated in HFREF patients. Taken together, these results suggest that reduction of myocardial interstitial collagen causes LV dilatation complicated with systolic dysfunction in the early stage of HFREF and that the increased myocardial interstitial collagen causes diastolic dysfunction in the advanced stage of HFREF with the resultant poor prognosis.

Although cardiac MRI is well established as a method for evaluating cardiac fibrosis, it cannot detect all cases of severe fibrosis, especially in HFREF patients with non-ischemic etiology.[28] It has also been reported that diffuse cardiac fibrosis is not able to be detected on cardiac MRI.[29] Furthermore, a recent study has shown that late gadolinium enhancement does not always indicate the change in myocardial interstitium.[30] Our preliminary data showed that there was no significant difference in CVF between the patients with and those without delayed enhancement on cardiac MRI (unpublished observation). Thus, we consider that the extent of myocardial fibrosis should be evaluated in multiple ways, including on endomyocardial biopsy, MRI and via serum markers of collagen turnover.

It has been reported that HFREF patients with diastolic dysfunction had a worse prognosis than those without it.[31,32] In the present study, elevated LVEDP was significantly related to increased CVF. Therefore, accumulation of myocardial interstitial fibrillar collagen may have caused ventricular diastolic dysfunction in the HFREF group with a resultant poor prognosis. The Randomized Aldactone Evaluation Study (RALES) showed that spironolactone improves prognosis in HF patients.[33] Interestingly, the RALES subanalysis showed that this benefit of spironolactone is noted only in patients with a high level of collagen synthesis marker (PIIINP) but not in those with low PIIINP.[11,33] It has also been shown that spironolactone reduced LV diastolic dysfunction only in DCM patients with increased myocardial fibrosis.[34]

In contrast, HFPEF seems to be a very different condition from HFREF in terms of response to medical treatment. Although ARB and ACEI could decrease myocardial fibrosis in HFPEF,[4,35,36] large clinical trials failed to demonstrate any beneficial effects of ARB or ACEI (eg, irbesartan, candesartan, enalapril, and valsartan) in patients with HFPEF.[37–40] This is consistent with the present finding that no significant correlation was noted between myocardial fibrosis and cardiac events in the HFPEF group, suggesting that the prognostic impact of myocardial fibrosis might be small in HFPEF. It has been previously reported, however, that in approximately 20% of patients with HFPEF, LVEF was significantly decreased during the 3-month follow-up period,[41] which is consistent with the present study, in which LVEF was significantly decreased in 11% of patients with HFPEF during follow-up. Thus, patients with severe myocardial fibrosis should be closely followed up because HFPEF patients with large CVF are at higher risk for disease progression and poor prognosis.

CVF and LVEDP

In the present study a significant but relatively weak correlation was noted between CVF and LVEDP in the HFREF group, probably because 60–70% of the HFREF patients received β-blockers and ACEI and/or ARB, which might have affected the systemic hemodynamics measured during cardiac catheterization.

As mentioned here, we were unable to observe any significant correlation between CVF and LVEDP in the HFPEF group, probably because the HFPEF is associated with heterogeneous diseases, such as hypertensive heart disease, cardiac amyloidosis, early-stage DCM and hypertrophic cardiomyopathy, and could also have been affected by the medical treatment including β-blocker and ACEI and/or ARB.

Study Limitations

Several limitations should be mentioned for the present study. First, we assessed only the collagen content in the myocardium. It was previously reported that not only the quantity but also the quality of collagen are important determinants for myocardial stiffness.[42] Indeed, the ratio of cross-linked collagen (insoluble collagen) to non-cross-linked collagen (soluble collagen) and the type I/type III collagen ratio are important determinants of myocardial stiffness,[17,27,43] and reduction in collagen cross-linking ameliorates myocardial stiffness and ventricular dilatation irrespective of collagen content.[44] Although we did not measure collagen turnover markers that have been established as prognostic in HF patients, it has been reported that there is a significant correlation between CVF and procollagen I carboxy-terminal peptide (PICP), a collagen synthesis marker.[45] Thus, the quality of ventricular fibrosis should be evaluated in biopsy specimens in future studies.

Second, because myocardial fibrosis may exist in a patchy fashion, we obtained at least 3 endomyocardial biopsy samples in each patient and evaluated CVF in as many fields as possible (mean, 3.6±0.9 fields) in order to minimize errors from patchy distribution of myocardial fibrosis in the present study. We still consider that we should evaluate the extent of myocardial fibrosis in multiple ways, including on endomyocardial biopsy, MRI and via serum markers of collagen turnover.

Third, in the present study, the HF subject group might be biased because we included patients who underwent endomyocardial biopsy alone and excluded those with other major causes of HF, such as ischemic heart disease and valvular heart disease. But because we did not include HF patients with valvular or ischemic etiology, we were able to minimize the overestimation of LVEF due to those factors in the present study.

Fourth, the present study was an observational study with a relatively small number of patients, and for reasons of ethics we were unable to perform repetitive myocardial biopsy to evaluate the time-course of HF. Thus, a future study with a large number of patients with a longer follow-up is required to address this issue.

Finally, the relatively small number of events limits the generalization of the present findings. Although we analyzed the present results with several statistical models, we found that the Cox proportional hazard model was the best. Thus, after univariate analysis, we used the Cox proportional hazard model with as small covariates as possible.

In conclusion, we have demonstrated that myocardial CVF evaluated with biopsy samples is a useful predictor for long-term survival in patients with HFREF (but not in those with HFPEF), and may be an important therapeutic target as well.

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Disclosures

None.

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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 –

Nobuyuki Shiba, MD, PhD; Kotaro Nochioka, MD, PhD; Masanobu Miura, MD; Haruka Kohno; Hiroaki Shimokawa, MD, PhD on behalf of the CHART-2 Investigators

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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Departments of Cardiovascular Medicine and Evidence-based Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan
The Guest Editor for this article was Masafumi Kitakaze, MD.
Mailing address: Nobuyuki Shiba, MD, PhD, Department of Evidence-based Cardiovascular Medicine and Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan. E-mail: nshiba@cardio.med.tohoku.ac.jp
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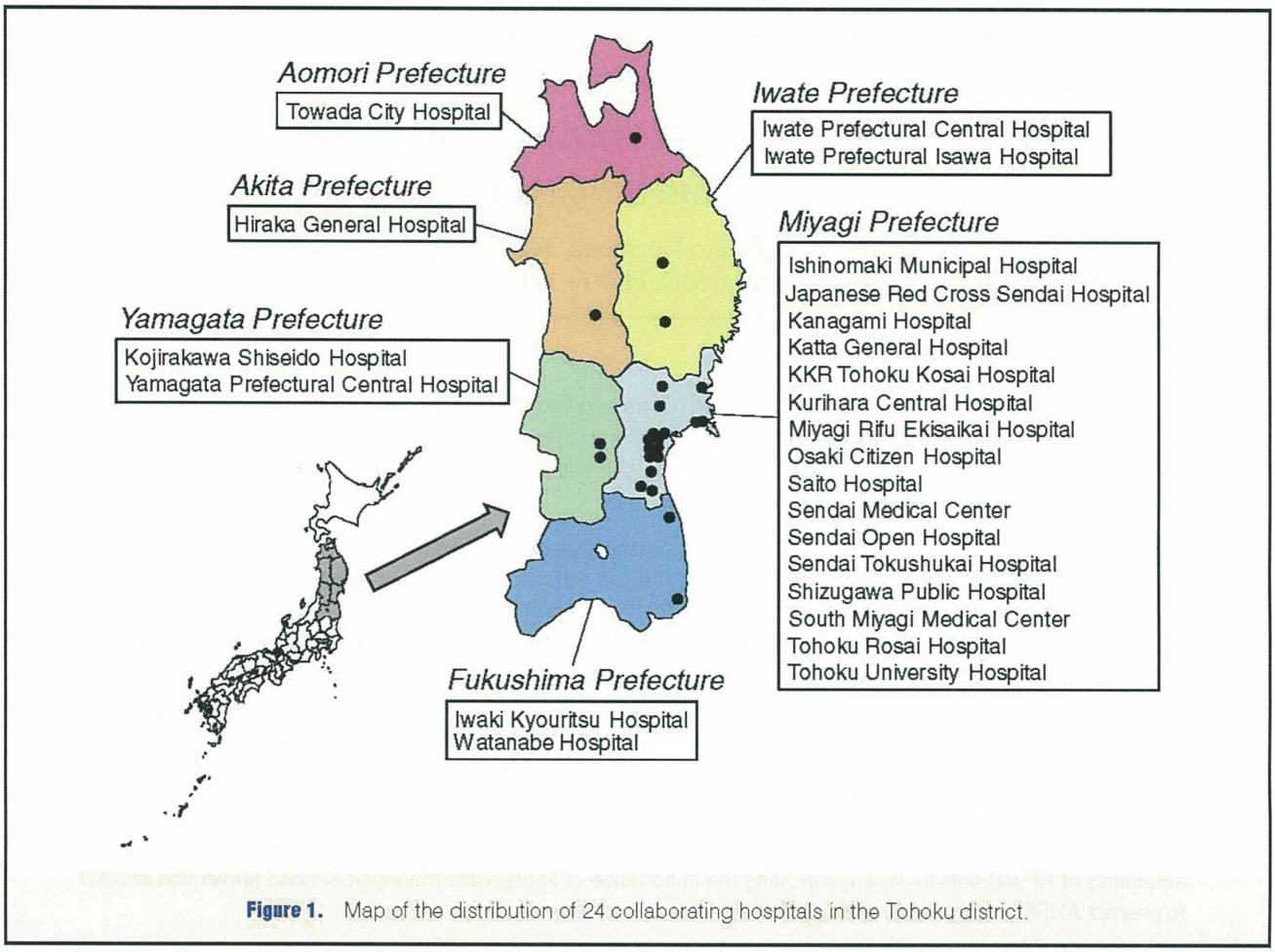

Figure 1. Map of the distribution of 24 collaborating hospitals in the Tohoku district.

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.

Editorial p775

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).

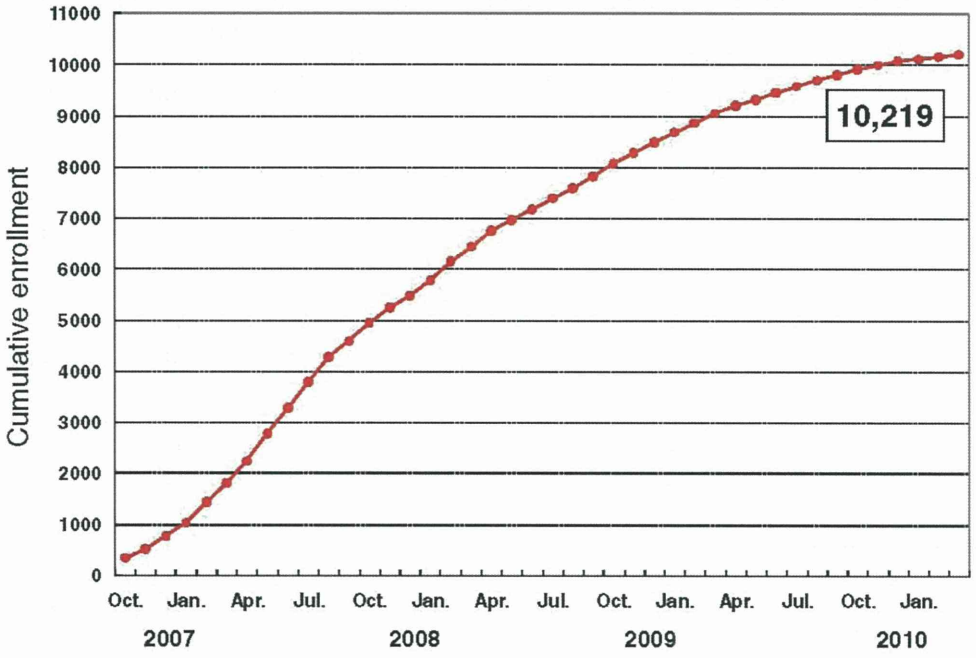

Figure 2. CHART-2 Study cumulative enrollment, showing a total of 10,219 registrations.

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.

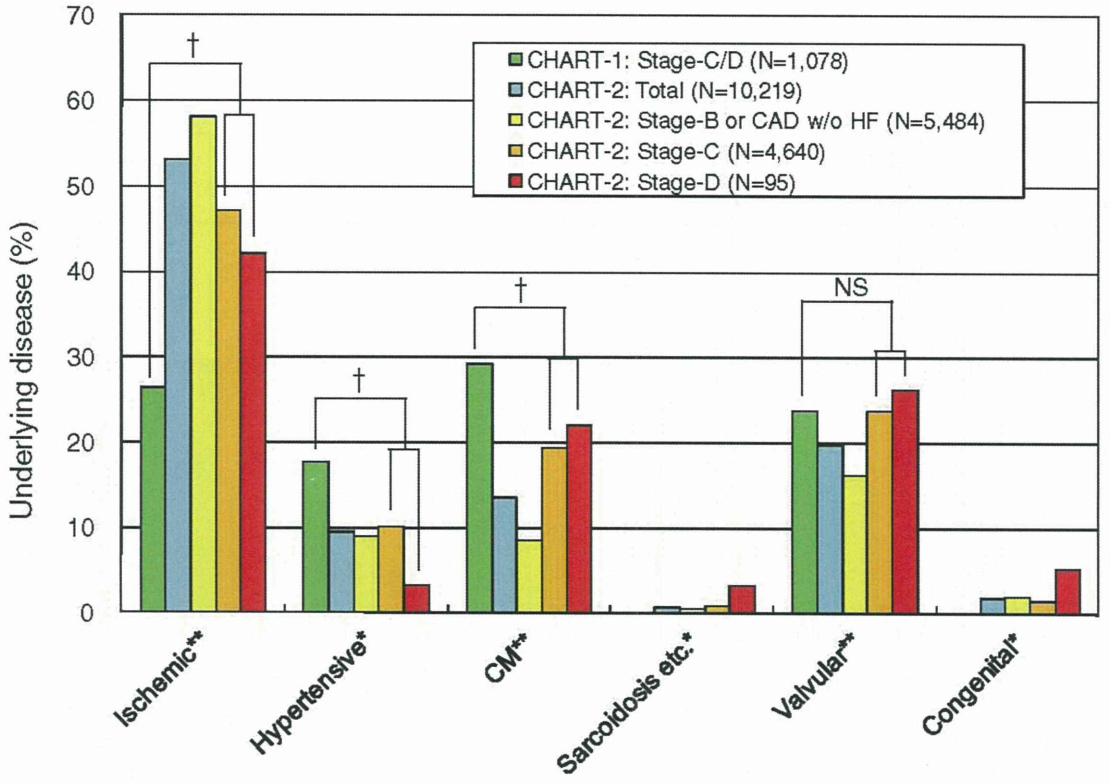

Figure 3. Underlying disease of the CHART-1 and the CHART-2 patients. CAD, coronary artery disease; CM, cardiomyopathy; HF, heart failure; w/o, without. *P<0.05 and **P<0.001 between patients in stage B/CAD, stage C, and stage D in the CHART-2 Study. †P<0.001 between stage C/D patients in the CHART-1 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.[4,5] 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

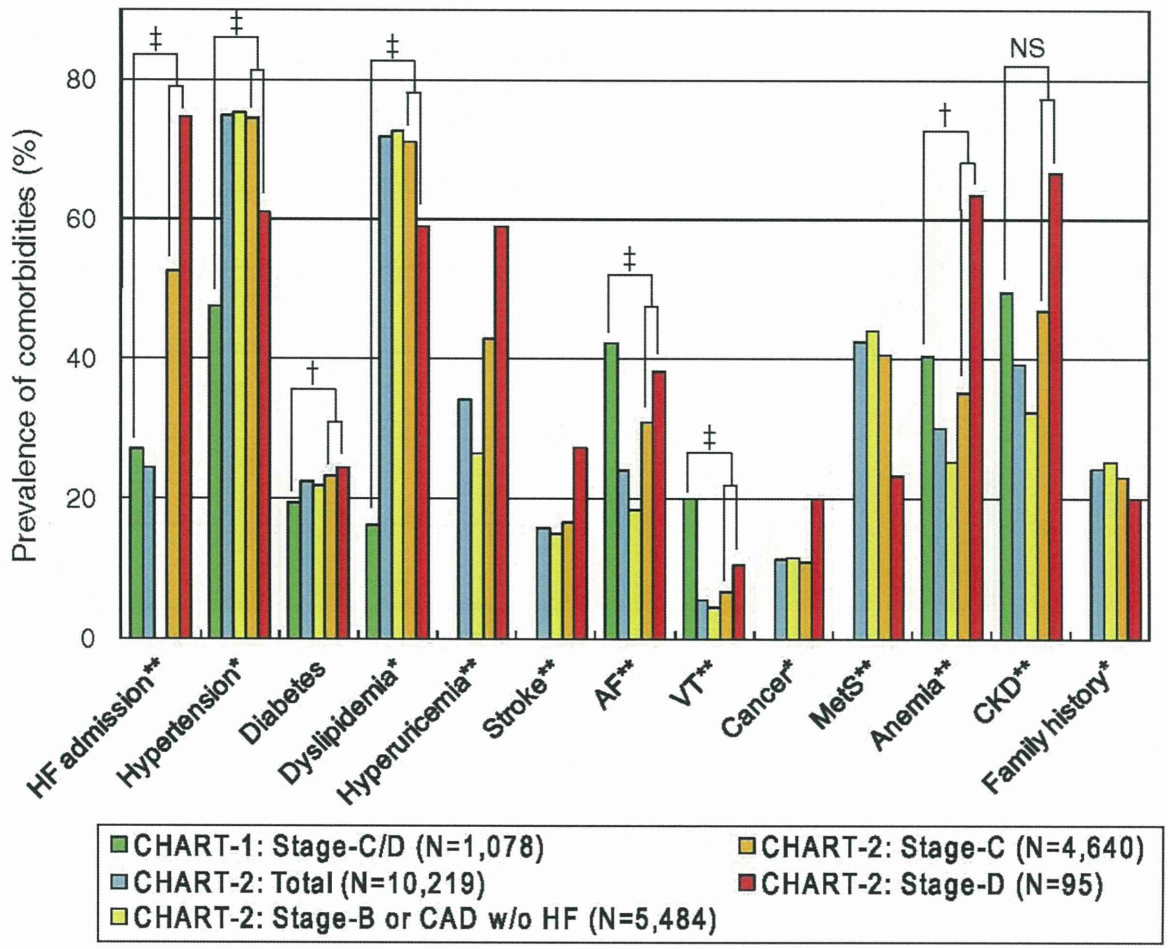

Figure 4. Comorbidities of the CHART-1 and the CHART-2 patients. AF, atrial fibrillation/flutter; CAD, coronary artery disease; CKD, chronic kidney disease; HF, heart failure; MetS, metabolic syndrome; VT, ventricular tachycardia; w/o, without. *P<0.05 and **P<0.001 between patients in stage B/CAD, stage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

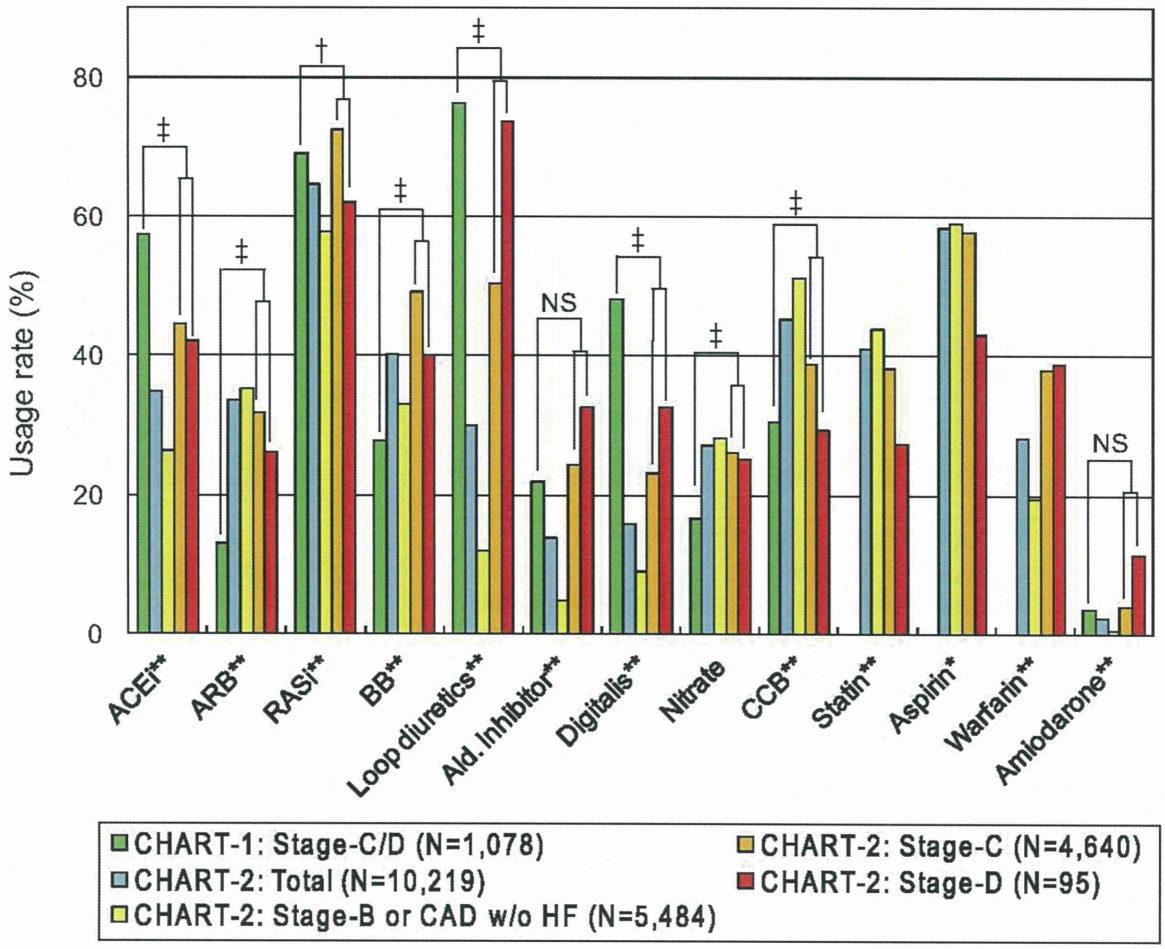

Figure 5. Medication of the CHART-1 and CHART-2 patients. ACEi, angiotensin-converting enzyme inhibitor; Ald., aldosterone; ARB, angiotensin II receptor blocker; BB, β-blocker; CAD, coronary artery disease; CCB, calcium channel blocker; RASi, renin–angiotensin system inhibitor; w/o, without. *P<0.05 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**).

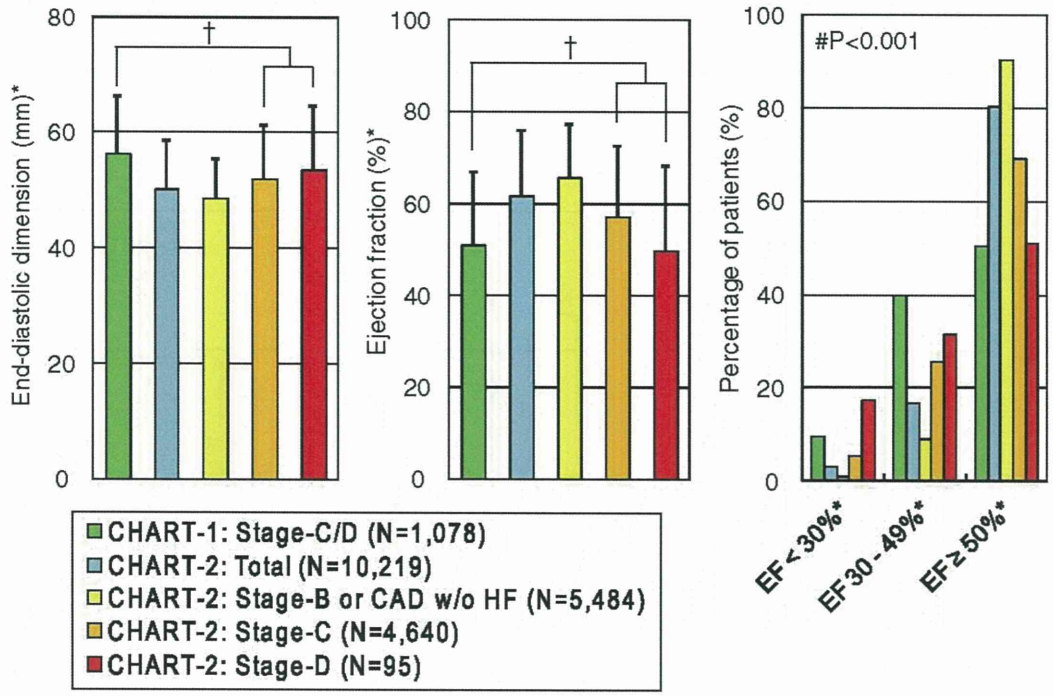

Figure 6. Left ventricular dimension and ejection fraction of the CHART-1 and CHART-2 patients. CAD, coronary artery disease; EF, ejection fraction. *P<0.001 between patients in stage B/CAD, stage C, and stage D in the CHART-2 Study. †P<0.001 between stage C/D patients in the CHART-1 Study and those 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)[8]	EuroHeart Failure Survey II (2006)[9]	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,[8,9,23] 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,[8,9,23] 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.[8,9,23,25] 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.[12,13,20] 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.

Acknowledgments

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Appendix 1

Study Organization of The CHART-2 Study

Executive Committee

Hiroaki Shimokawa (Chair), Mitsumasa Fukuchi, Toshikazu Goto, Tetsuya Hiramoto, Kanichi Inoue, Atsushi Kato, Tatsuya Komaru, Masatoshi Ohe, Nobuyo Sekiguchi, Nobuyuki Shiba, Tsuyoshi Shinozaki, Masafumi Sugi, Kenji Tamaki.

Steering Committee

Tetsuya Hiramoto, Kanichi Inoue, Atsushi Kato, Masahiko Ogata, Syoichi Sato, Masafumi Sugi.

Collaborating Hospitals and Active Investigators by Prefecture

Aomori Prefecture

J. Kikuchi, S. Oyama, T. Tajima (Towada City Hospital).*

Iwate Prefecture

K. Tamaki, E. Nozaki, N. Hoshi (Iwate Prefectural Central Hospital).*
M. Nakagawa (Iwate Prefectural Isawa Hospital).*

Akita Prefecture

M. Hayashi, N. Sekiguchi, Y. Sugai, Y. Asaumi, K. Fukahori, S. Takeda, H. Endo, Y. Kokusho (Hiraka General Hospital).*

Yamagata Prefecture

M. Ohe, T. Kobayashi, M. Morita, K. Sakurai, T. Kagatani, A. Sato (Kojirakawa Shiseido Hospital).*
T. Goto, T. Yahagi, M. Matsui, Y. Tamada, A. Fukui, K. Takahashi, K. Takahashi, K. Oumi, K. Hao, S. Sasaki, A. Kikuchi, S. Suzuki, N. Yaoita (Yamagata Prefectural Central Hospital).

Miyagi Prefecture

K. Akai, Y. Konno, J. Demachi, T. Tajima, N. Takahashi (Ishinomaki Municipal Hospital).
Y. Konno (Japanese Red Cross Sendai Hospital).
N. Shiba, M. Wakayama (Kanagami Hospital).
H. Kanno, T. Ishizuka, J. Kaneko (Katta General Hospital).*
M. Fukuchi, N. Shiba, A. Sugimura, J. Otomo, H. Tada, Y. Ito (KKR Tohoku Kosai Hospital)*.
S. Komatsu, J. Suzuki (Kurihara Central Hospital).
D. Katayose (Miyagi Rifu Ekisaikai Hospital).*
S. Onodera, T. Hiramoto, M. Chida, K. Iwabuchi, M. Takeuchi, M. Takeda, K. Yahagi (Osaki Citizen Hospital).
Y. Kagaya, K. Otsuka, K. Komaki, Y. Koseki (Saito Hospital).
T. Shinozaki, T. Tanikawa, S. Baba, T. Tomioka, M. Tanaka, N. Onoue (Sendai Medical Center).*
M. Kanazawa, A. Kato, S. Namiuchi, B. H. Ong, C. Takahashi, T. Sugie, H. Tada (Sendai Open Hospital).*
S. Sato, M. Fukuchi, M. Ogata, K. Sakurai, A. Sugimura, T. Tomioka, O. Kitamukai (Sendai Tokushukai Hospital).*
Y. Fukumoto (Shizugawa Public Hospital).
K. Inoue, S. Horiguchi, J. Koyama, H. Shioiri, T. Tomioka (South Miyagi Medical Center).
T. Sakuma, T. Komaru, H. Kato, K. Saji (Tohoku Rosai Hospital)*.
Y. Kagaya, M. Miura, T. Komaru, N. Shiba, S. Yasuda, A. Karibe, J. Demachi, Y. Fukumoto, J. Nawata, K. Ito, M. Nakayama, K. Fukuda, M. Takeda, Y. Wakayama, R. Koshida, J. Takahashi, M. Wakayama, K. Kumagai, Y. Sugai, K. Sugimura, M. Hirose, K. Satoh, Y. Asaumi, H. Tada, K. Saji, T. Tada, M. Nakano, Y. Kokusho, S. Fukui, N. Yamaguchi, Y. Ito, R. Tsuburaya, K. Nochioka, Y. Miura, J. Ohashi, K. Aizawa, Y. Kikuchi, T. Takii, M. Kondo, K. Hao, K. Takahashi, Y. Takagi, S. Nakajima, S. Tatebe, T. Shimizu, S. Miyamichi, K. Noda (Tohoku University Hospital).*

Fukushima Prefecture

M. Sugi, Y. Yamamoto, Y. Minatoya, T. Tada, T. Shiroto (Iwaki Kyouritsu Hospital).*
K. Fukuda, M. Takeda (Watanabe Hospital).*
*Fifteen hospitals were collaborating institutions in the CHART-1 Study.

Head Office and Coordinating Center

N. Shiba, K. Nochioka, M. Miura, M. Matsuki, S. Osaki, D. Ito, K. Yukishita, Y. Kidoguchi, M. Hotta, H. Kohno, Y. Nakajima, E. Yanagisawa, A. Mori, M. Takahashi, R. Takeuchi, K. Takahashi, S. Toudera, M. Sugaya, M. Sato, C. Saga, J. Suenaga, M. Kikuchi, F. Mori.

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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REVIEW

Clinical update

Fish oil and omega-3 fatty acids in cardiovascular disease: do they really work?

Daan Kromhout[1]*, Satoshi Yasuda[2], Johanna M. Geleijnse[1], and Hiroaki Shimokawa[2]*

[1]Division of Human Nutrition, Wageningen University, PO Box 8129, 6700 EV Wageningen, The Netherlands; and [2]Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan

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Omega-3 fatty acids, which are found abundantly in fish oil, exert pleiotropic cardiometabolic effects with a diverse range of actions. The results of previous studies raised a lot of interest in the role of fish oil and omega-3 fatty acids in primary and secondary prevention of cardiovascular diseases. The present review will focus on the current clinical uses of omega-3 fatty acids and provide an update on their effects. Since recently published trials in patients with coronary artery diseases or post-myocardial infarction did not show an effect of omega-3 fatty acids on major cardiovascular endpoints, this review will examine the limitations of those data and suggest recommendations for the use of omega-3 fatty acids.

Keywords Fish oils • Cardiovascular disease

Introduction

In 1929, the essential fatty acids were discovered by the biochemists Evans and Burr.[1] They showed that mammals do not possess enzymes able to synthesize double bonds at the *n*-3 and *n*-6 positions of the carbon chain of a fatty acid. Therefore, humans must obtain the essential fatty acids linoleic acid (C18:2*n*-6) and alpha linolenic acid (ALA, C18:3*n*-3) from dietary sources. Alpha linolenic acid can be extended to eicosapentaenoic acid (EPA C20:5*n*-3) and docosahexaenoic acid (DHA C22:6*n*-3) through elongation and desaturation. Fish oil is a rich source of these omega-3 fatty acids.

In 1937, the British physiologist Hugh Sinclair visited Evans and became interested in the possibility that deficiencies in polyunsaturated fatty acids could cause coronary artery diseases (CAD). In 1944, he undertook his first visit to the Inuit and became convinced that their diet protects against atherosclerosis and Western diseases.[2] In a letter to the *Lancet*, he hypothesized in 1956 that omega-3 fatty acids may be responsible for the protective effect of their diet.[3] This view was contrary to the dogma of that time that all animal fats are harmful. In the 1970s, he joined the Danish investigators Bang and Dyerberg[4,5] during one of their expeditions to Greenland. They found that the Inuit consumed ~400 g of seafood per day and their average intake of omega-3 fatty acids was 14 g per day compared with 3 g per day among Danes. An epidemiological study showed that the incidence of myocardial infarction (MI) was 10 times lower among the Inuit compared with the Danes.[6]

The difference between the Inuit and the Danes in the intake of omega-3 fatty acids was reflected in their fatty acid composition of platelets. Differences were also observed in haemostatic factors, bleeding time, serum triglycerides, and high-density lipoprotein (HDL)—cholesterol levels. To show that these associations are causal, Sinclair put himself in 1977 on an Inuit diet for 100 days.[7] His bleeding time rose from 3–5 to 50 min and substantial decreases were observed in blood platelets, erythrocytes, packed cell volume, and haemoglobin. The triglyceride-rich very low-density lipoprotein (VLDL) fell and the HDL fraction increased considerably. A substantial increase in the EPA concentration and a marked decrease in the linoleic acid concentration of cholesteryl esters were noted. Sinclair concluded from this experiment that it is necessary to have the right balance of omega-3 and omega-6 fatty acids to prevent thrombotic disorders.

In 1985, Kromhout *et al.*[8] showed in the Zutphen Study, a prospective cohort study in the Netherlands, that eating fish once or twice per week was associated with a lower risk of fatal CAD

* Corresponding authors: Tel: +31 317 483054, Fax: +31 317 483342, Email: daan.kromhout@wur.nl (D.K.)/Tel: +81 22 717 7151, Fax: +81 22 717 7156, Email: shimo@cardio.med.tohoku.ac.jp (H.S.)