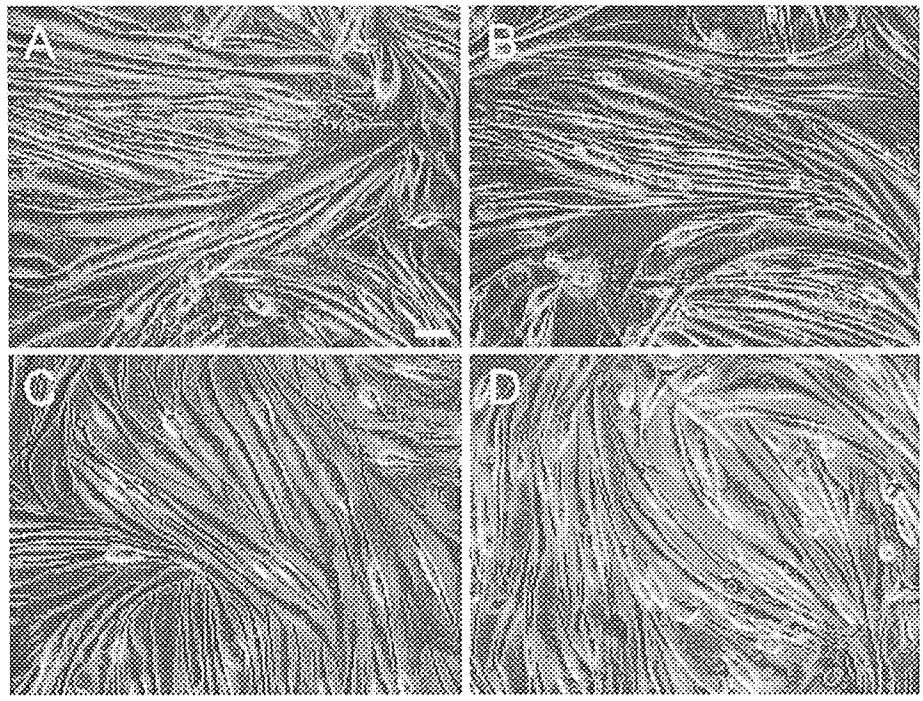

Fig. 6 Prevention of myotube formation by inhibition of calpain activity. Cells transfected with Ad_SCR (SCR) or Ad_δ-SG (δ-SG) were cultured in GM for 3 days. Three days after transfection, C_2C_{12} myoblasts were re-infected with Ad_shSCR (**A**, **C**) or Ad_shcapn2 (**B**, **D**) and the medium was replaced by DM [19]. On day 10, these cells were examined with light microscopy. All images were observed at the same magnification, and the bar indicates 20 μm

other SGs without mechanical stress. These results indicate that mechanical stress is not a primary cause for the loss of other SGs when δ-SG gene was deficient. On the other hand, previous studies have reported that mechanical stress such as muscle stretch [29–32], exercise [33], or contraction [32, 34] affected muscle damage in SG-deficient animals or MD and/or CM animals. In addition, both in vitro and in vivo studies have shown that SG-deficient muscle cells are vulnerable to membrane damage when subjected to the mechanical stress [30]. These results imply that mechanical stress is one of the key factors to promote membrane fragility and instability in SG-deficient cells. It is conceivable that mechanical stress would accelerate muscular degeneration.

We also found that each level of α-, β-, and γ-SG protein decreased in δ-SG knockdown myotubes, although the transcription of these SGs was preserved. These data are consistent with those from animal models [6, 11] and suggest that α-, β-, and γ-SG are degraded after the translation. So far, three pathways have been reported for protein catabolism in myotubes: (1) hydrolysis by lysosomal proteases, (2) cytosolic calpains, and (3) non-lysosomal ATP-ubiquitin- dependent proteases. Previous studies have reported that defects in SG and concomitant loss of DAP complex resulted in mechanical instability of the SL [35] and increased Ca^{2+} permeability causing a chronic rise in the intracellular Ca^{2+} [36–38]. The subsequent elevation of cytosolic Ca^{2+}-sensitive proteases, calpains included in the cytosolic fraction, and activated calpain may accelerate the proteolysis. Iwata's group clearly showed that the resting level of intracellular Ca^{2+} was significantly higher in BIO14.6 myotubes than in those from normal controls [36, 37]. These results indicate that myotubes from BIO14.6 hamsters show abnormal intracellular Ca^{2+} homeostasis even at the resting state, and high $[Ca^{2+}]_i$ in δ-SG knockdown cells triggers the activation of calpain and the subsequent degradation of DAP complex.

Previous study has also demonstrated that basal calpain activity was elevated in SG-deficient SARCAFI mice [39]. In addition, α-, β-, and γ-SG and Dys are preferentially hydrolyzed by calpain in vitro [40, 41]. These data suggest that an enhanced proteolysis particularly with calpain is a key factor in the progression of MD and/or CM.

On the other hand, recent studies indicate that the involvement of proteasome activity in the pathogenesis of LGMD-2D and demonstrate that interfering with this activity promotes the correct localization of the disease-causing α-SG mutants [13]. And the efficient block of degradation rescued the expression and localization of Dys and DAPs. However, in these investigations, inhibitors were treated for short term but could not completely recover DAPs. Therefore, more researches are necessary to define the proteolytic process involved in calpain and/or ubiquitin–proteasome system. In conclusion, the specific knockdown system employed here may help us to clarify the mechanism of progression in MD and/or DCM [42].

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Chronic heart failure in Japan: Implications of the CHART studies

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Abstract: The prognosis of patients with chronic heart failure (CHF) still remains poor, despite the recent advances in medical and surgical treatment. Furthermore, CHF is a major public health problem in most industrialized countries where the elderly population is rapidly increasing. Although the prevalence and mortality of CHF used to be relatively low in Japan, the disorder has been markedly increasing due to the rapid aging of the society and the Westernization of lifestyle that facilitates the development of coronary artery disease. The Chronic Heart Failure Analysis and Registry in the Tohoku District (CHART)-1 study was one of the largest cohorts in Japan. The study has clarified the characteristics and prognosis of Japanese patients with CHF, demonstrating that their prognosis was similarly poor compared with those in Western countries. However, we still need evidence for the prevention and treatment of CHF based on the large cohort studies or randomized treatment trials in the Japanese population. Since the strategy for CHF management is now changing from treatment to prevention, a larger-size prospective cohort, called the CHART-2 study, has been initiated to evaluate the risk factors of CHF in Japan. This review summarizes the current status of CHF studies in Japan and discusses their future perspectives.

Keywords: heart failure, aging, Japanese

Introduction

Chronic heart failure (CHF) is the leading cause of mortality in most developed countries (Hunt et al 2001). The prevalence and mortality rates of CHF used to be relatively lower in Japan compared with other Western countries. In Japan, approximately 1 to 2 million patients have CHF and nearly 170,000 patients die due to heart diseases each year (approximately 130 per 100,000 person-years) (Summary of Vital Statistics 2005). However, the prevalence and death rates of cardiovascular diseases and CHF have been rapidly increasing in Japan, due to the Westernization of lifestyle, including dietary habits, and the aging population (The Status of Aging 2007). The Chronic Heart Failure Analysis and Registry in the Tohoku District (CHART)-1 study was one of the largest cohort studies with Japanese CHF patients (N = 1,278), which was designed to evaluate the characteristics and prognosis of those patients. We have also started a new cohort study, named the CHART-2 study (N = 10,000, expected) aiming to elucidate the effective preventive measures for CHF. This review briefly summarizes the major socio-medical issues of Japanese patients with CHF, their clinical characteristics and prognosis found in our CHART-1 study, and the current status of CHF studies in Japan.

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Socio-medical status of Japanese patients with CHF

Rapid aging of Japanese population

Until the 1980s, Japan had a lower percentage of elderly citizens compared with any other developed countries. However Japan is now one of the countries in which the

population is aging rapidly. Figure 1 shows the time-course of aging and population projections between 1950 and 2055, which is assembled using the data reported by the Japanese Cabinet Office (The Status of Aging 2007). As of October 1, 2006, the total population of Japan was 127.8 million and the number of elderly aged 65 or older was 26.6 million, accounting for 21% of the total population. The elderly population is expected to continue to increase rapidly and the percentage of the elderly will reach 35.8 million (27%) in 2055 (The Status of Aging 2007). Life expectancy in Japan at birth has also drastically increased since World War II to 78.6 years for males and 85.5 years for females in 2005 (Life Expectancies at Specified Ages 2006). The Japanese Cabinet Office expects that it will reach 83.7 years for males and 90.3 years for female in 2055. Thus, in the near future, Japanese society will encounter more difficult medical problems due to rapid aging, which other developed countries have never before experienced.

Changing causes of death in Japan

Infectious diseases such as pneumonia, tuberculosis, and gastroenteritis were the leading causes of death in Japan until the mid 1900s. The major health problems in Japanese society have drastically changed since World War II. The morbidity and mortality rates of lifestyle-related diseases such as cancer, heart disease, stroke, and diabetes mellitus have dramatically increased. Approximately 60% of the mortality is now attributed to lifestyle-related diseases (cancer, 31%; ischemic heart disease 16%; cerebrovascular disease 13%; diabetes mellitus 1%; and hypertensive disease 0.6%) and the medical costs for these diseases amounts to 10.2 trillion yen (87.8 billion US dollars), accounting for approximately 30% of the total cost of the Japanese health insurance in 2003 (Exercise and Physical Activity Reference for Health Promotion 2006). Currently, heart disease is the second most frequent cause of death in Japan. Figure 1 shows the trend of the mortality due to heart diseases, which is constructed using the reports of death certificates in Japan (Summary of Vital Statistics 2005). There is a clear trend for the increase in death due to heart disease since 1950s (there was a temporary sharp decline in 1995 due to the tenth revision of the International Classification of Diseases regarding the description of diagnosis in death certificates).

Health insurance system and future economic burden in Japan

In Japan, all citizens are enrolled in the mandatory health insurance system based on employment and residential status. The average number of visits to a doctor per year is 16 in Japan, versus 5.8 visits in the United States (Itoh 2004). As elderly patients tend to visit doctors more frequently and to have more medication or high-cost medical care, medical

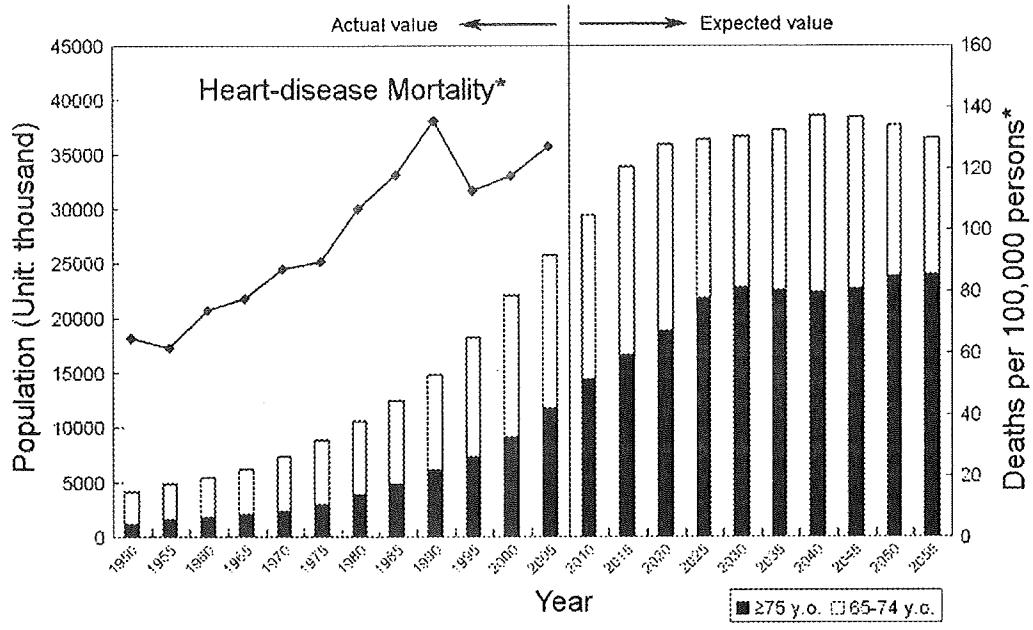


Figure 1 Time-course and future prediction of the increase in elderly population and heart-disease mortality in Japan. Data are based on the Status of Aging and Implementation of Measures for Aging Society in 2005, reported by the Japanese Cabinet.

expenditure for the elderly is already taking one-third of the national health expenditure, and is projected to reach a half of the expenditure by the year 2025 (Itoh 2004). Progressive aging of the society and the consequent increase in the number of patients with CHF will cause more financial burden within Japanese society, which could lower the quality of medical services in the future.

An overview of heart failure studies in Japan

In Japan, medical treatment for patients with CHF is mainly based on the evidence obtained from randomized trials in the United States and Europe. There have been no sufficient randomized treatment trials or prospective cohort studies in Japan to clarify the real characteristics of Japanese patients with CHF or to improve their prognosis and quality of life. The mandatory health insurance system, the shortage of the budget to fulfill mega-trials, and the absence of trained research nurse system may all be responsible for the current situation. This section describes several cohort studies with Japanese CHF patients, major outcomes of the CHART-1 study, and randomized treatment trials for CHF performed with Japanese patients, either those that have already been published or are currently in progress. Finally, racial differences will be discussed, because this issue may also influence the impact of risk factors and/or the effects of treatments for CHF.

Prospective cohort studies in Japan

There are few multi-institutional prospective cohort studies with CHF patients in Japan (Table 1). The Chronic Heart Failure Analysis and Registry in the Tohoku District (CHART)-1 study was the first cohort study in Japan, including more than 1,000 Japanese patients with stable CHF, who were registered at 26 hospitals in the Tohoku district with a population of approximately 9.8 million (Shiba et al 2004). The CHART-1 study was initiated in February 2000 and was completed in December 2005. The total number of CHF patients enrolled was 1,278 and the mean follow-up period was 3.5 years. Details of design and the main outcome will be presented at the following part in this article. The Japanese Cardiac Registry of Heart Failure in Cardiology (JCARE-CARD) is a registry of hospitalized patients with worsening CHF at 164 hospitals throughout the country between January 2004 and June 2005 (Tsutsui et al 2006). Death and hospital admission of the patients were followed through 2006 with the mean follow-up period of at least 1 year. Results of this study will appear in the near future. The Japanese Cardiac Registry of

Table 1 Multicenter prospective cohort study for patients with chronic heart failure in Japan

Study [Reference]	Study population	Age, years (Mean)	Total enrollment	Heart failure stage/NYHA	Study start Expected completion	Mean follow-up	1-year mortality	Status
CHART-1 Study [Shiba 2004]	Chronic heart failure outpatients/ hospitalized pts.	≥18 (68)	1278	(B) C-D I-IV		3.5 years	7.3%	Published
JCARE-CARD [Tsutsui 2006]	Hospitalized patients with heart failure	≥15	2676	C-D		At least one year		Completed
JCARE-GENERAL [Tsutsui 2007]	Outpatients with heart failure	≥15 (74)	2685	C-D		One year	6.3%	Published
CHART-2 Study	Chronic heart failure	≥20	10000	B-D	Oct 2006			Recruiting
	High risk for heart failure outpatients/ hospitalized pts.		(expected)	I-IV	Sep 2011			

Data are retrieved from published papers or the UMIN Clinical Trials Registry (http://www.umin.ac.jp/ctr/index-j.htm)/ClinicalTrials.gov (http://clinicaltrials.gov/).
Abbreviations: NYHA, New York Heart Association; pts, patients.

Heart Failure in General Practice (JCARE-GENERAL) is a registry of outpatients with CHF managed by cardiologists in hospitals and primary care physicians in general practice (Tsutsui et al 2007). Baseline data of totally 2,685 patients were collected during November 2004 and follow-up data were collected for 1 year after the enrolment. During the mean follow-up period of 427 days, the crude mortality rate was 6.7% in patients managed by cardiologists and 5.9% in those managed by general physicians. The Chronic Heart Failure Analysis and Registry in the Tohoku District (CHART)-2 study is currently the largest prospective and hospital-based cohort study with patients with CHF in Japan. This study was designed to investigate the characteristics and prognosis of a total of 10,000 patients with symptomatic CHF (Stage C/D in the ACC/AHA classification) and those with structural heart disease but without signs or symptoms of CHF (Stage-B in the AHA/ACC classification) (Hunt et al 2001). This study will elucidate the incidence and prognostic impact of metabolic syndrome in those patients, especially on the development of the first symptomatic CHF. The CHART-2 study was started in October 2006 and will be completed in September 2011.

The CHART-1 Study

Risk stratification is the first line strategy to improve the prognosis and quality of life of patients with CHF. A number of factors have been found to correlate with the mortality of patients with CHF (Rector et al 1994; Deedwania 2003; Bettencourt et al 2000). The CHART-1 study was started to register patients with stable CHF in February 2000 to clarify the characteristics and prognosis and to seek for prognostic factors in Japanese CHF patients (Shiba et al 2004, 2005). Patients were enrolled when at least one of the following criteria was met: (1) left ventricular ejection fraction (LVEF) <50%, (2) left ventricular end-diastolic dimension >55 mm, or (3) at least one episode of congestive heart failure. Patients less than 18 years old or those with clinically unstable conditions were excluded. Baseline data, including laboratory findings, results of echocardiography, and medical treatments for CHF, were recorded and annual surveillance was performed until the end of 2005.

Characteristics and prognosis of patients with CHF in Japan.

A total of 1,278 patients were enrolled in the CHART-1 cohort. The mean age of the study population was 68.3 years, and male accounted for 66% of the total study population. The prevalence of diabetes mellitus and hypertension was 19% and 47%, respectively. Other baseline characteristics of patients are shown in Table 2. Ischemic etiology accounted for only 25% and the percentage of patients older than 65 years was 66%. Patients with preserved systolic function (defined as LVEF >50%) accounted for 45% of the total population. During the mean follow-up period of 3.5 years, all-cause mortality rate at 1-, 2-, and 3-year was 7%, 16%, and 22%, respectively (Figure 2). Multivariate Cox analysis showed that several covariates, such as age, diabetes mellitus, ventricular tachycardia, serum level of B-type natriuretic peptide (BNP), rural residence, and NYHA functional class, were significantly associated with all-cause mortality (Shiba et al 2004). Figure 3 shows the Kaplan-Meier analyses of freedom from all-cause mortality in patients stratified by serum level of BNP or LVEF. Patients with higher BNP concentration had a significantly poorer prognosis (Watanabe et al 2005), however, the prognostic impact was not significantly different between patients with 200–500 pg/mL of BNP level and those with >500 pg/mL (Figure 3A). The all-cause mortality of patients with preserved systolic function (LVEF > 50%) was not significantly different than that of patients with

Table 2 Baseline characteristics of the Japanese patients in the CHART-1 study

No. of patients	1,278
Follow-up period (years)	3.5 ± 1.7
Age (years)	68.3 ± 13.4
≤39	3.7%
40–64	30.2%
65–74	32.8%
≥75	33.3%
Male (%)	66.0%
NYHA	
I	19.7%
II	63.0%
III	16.5%
IV	0.8%
Underlying disease	
Coronary artery disease	25.4%
Valvular heart disease	26.4%
Left ventricular hypertrophy	14.0%
Non-ischemic cardiomyopathy	28.6%
Other	5.6%
Left ventricular ejection fraction (%)	51.1 ± 15.9
<30%	11.7%
30–50%	43.7%
>50%	44.6%
Hypertension	47.4%
Diabetes	18.9%
Dyslipidemia	16.7%
Atrial fibrillation	41.8%
Ventricular tachycardia	20.1%
History of heart failure admission	23.4%

Abbreviations: NYHA, New York Heart Association.

moderately decreased LVEF (30%–50%). However the prognosis of those with severely low LVEF (<30%) was the lowest with frequent episodes of sudden cardiac death (Figure 3B). The 3-year incidence of sudden death was higher in patients with LVEF <30% than those with LVEF ≥30% (15% vs 4%, respectively, $p < 0.001$). Primary prevention of sudden cardiac death with an implantable cardioverter defibrillator in those patients should be recommended when they meet the criteria in the authorized guidelines (Watanabe et al 2006). Recently, anemia has been emphasized as an important prognostic predictor in patients with CHF (Ezekowitz et al 2003). Our data also showed that anemia was significantly associated with all-cause mortality, cardiac-cause mortality, and sudden death in patients with diastolic CHF (Tada et al 2007), as well as in those with systolic CHF, as reported by other researchers (O'Meara et al 2006).

Treatment of patients with CHF in Japan

Treatments with angiotensin converting enzyme inhibitors (ACEI), angiotensin II receptor blockers (ARB), or β-blockers are recommended to improve prognosis and quality of life for patients with CHF (Hunt et al 2001). However it has previously been reported that such evidence-based treatments might not be sufficiently used in patients who should have had benefits of such medications (Masoudi et al 2003). The overall usage rate of ACEI/ARB or β-blocker in patients enrolled in the CHART-1 study was 70% and 28%, respectively (Figure 4). The penetration rate of these medications was relatively lower in female patients, elderly patients, and those with valvular heart disease or preserved LVEF, and this trend was more evident for the treatment with β-blocker than ACEI/ARB (Figure 4). These results suggest that future clinical trials are still necessary for such minorities who have not usually been enrolled in major randomized treatment trials for CHF.

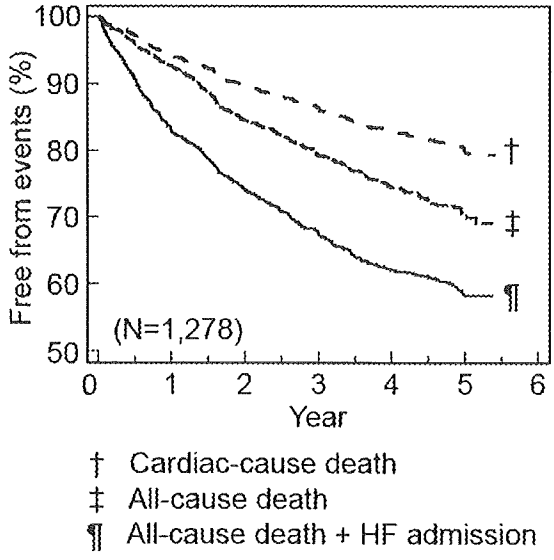


Figure 2 Prognosis of the Japanese patients with CHF in the CHART-1 study. Copyright © 2004. Reproduced with permission from Shiba N, Watanabe J, Shinozaki T, et al. 2004. Analysis of chronic heart failure registry in the Tohoku district: third year follow-up. *Circ J*, 68:427–34.
Abbreviations: HF, heart failure.

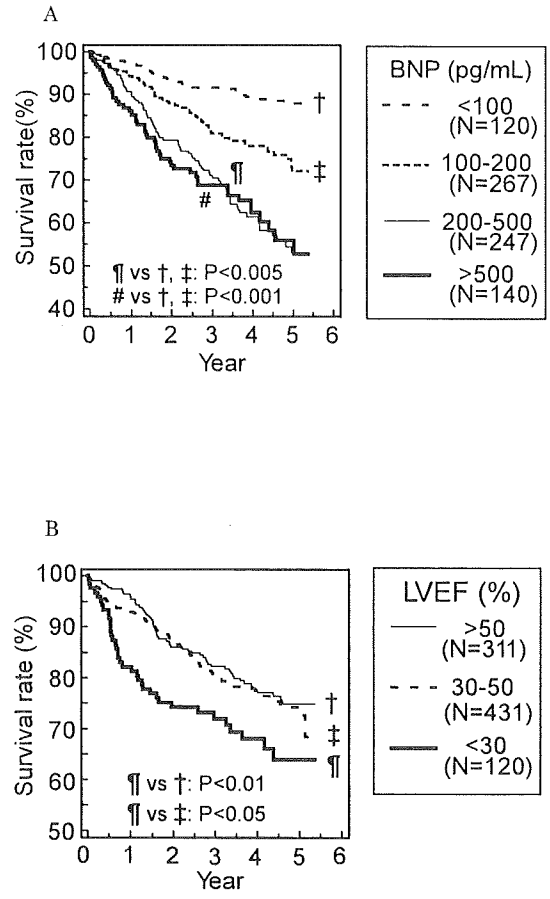


Figure 3 Kaplan-Meier curves of freedom from all-cause death stratified by **(A)** BNP and **(B)** LVEF in the CHART-1 study.
Abbreviations: BNP, B-type natriuretic peptide; LVEF, left ventricular ejection fraction.

Clinical outcomes of Japanese patients with CHF

Figure 5 showed survival curves of placebo groups in randomized treatment trials for CHF performed in Western countries, superimposed with the result obtained in our CHART-1 study with Japanese CHF patients. One-year all-cause mortality of patients with mild-moderate CHF (NYHA

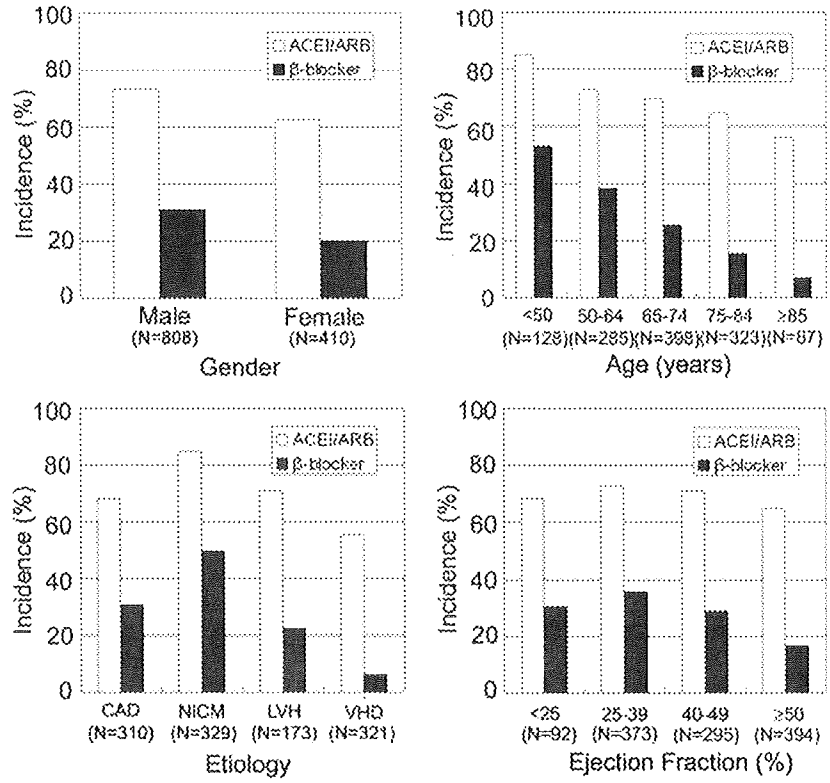


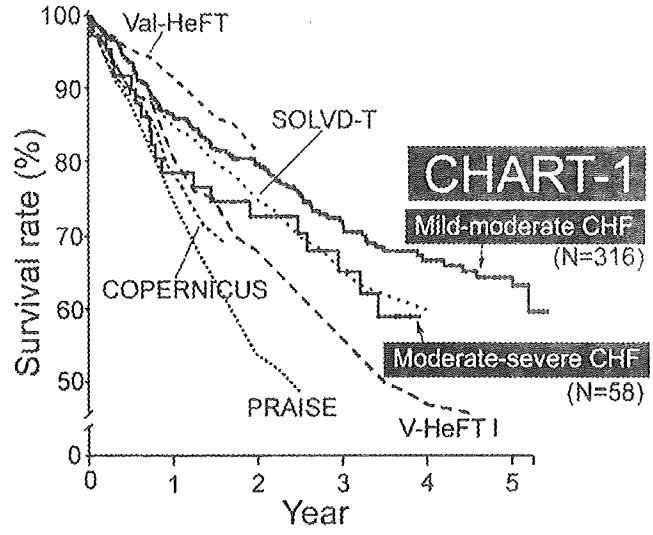
Figure 4 Prevalence of the use of renin-angiotensin inhibitors and β-blockers in the CHART-1 study. Copyright © 2007. Reproduced with permission from Shiba N, Takahashi J, Matsuki M. 2007. The CHART Study (Japanese). *Naika*, 99:410–14.
Abbreviations: ACEI, angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor blocker; CAD, coronary artery disease; NICM, non-ischemic cardiomyopathy; LVH, left ventricular hypertrophy; VHD, valvular heart disease.

II–IV and LVEF ≤40%) or moderate-severe CHF (NYHA III–IV and LVEF ≤35%) was 13% and 21%, respectively. Importantly, as is evident in Figure 5, the prognosis of Japanese patients with CHF was equally poor compared with Western CHF patients. Since the Japanese society is aging rapidly, a sharp increase in the number of CHF patients will be inevitable in the near future in Japan, as CHF is a disease of the elderly. Figure 6 shows the event rate in CHF patients based on age at the entry, demonstrating that elderly Japanese patients with CHF had an increased incidence of cardiac death and a combination of cardiac death and admission due to congestive CHF. This is because elderly CHF patients have a higher rate of combined risk factors, such as anemia, chronic kidney disease, hypertension, and atrial fibrillation. Appropriate prevention strategies against the development and progression of CHF should be undertaken in Japan.

Major CHF treatment trials in Japan

There are 2 published randomized treatment trials for CHF patients in Japan (Table 3). The Multicenter Carvedilol Heart Failure Dose Assessment (MUCHA) trial enrolled 174 patients with mild to moderate CHF to seek for the efficacy and optimum dose of carvedilol, with 3 treatment arms, including placebo, 5 and 20 mg of the β-blocker in daily dose (Hori et al 2004). During the 24–48 weeks of the treatment period, carvedilol achieved dose-related improvement of the rate of death or cardiovascular hospitalization to 25%, 9%, and 5% in the placebo, 5 mg, and 20 mg group, respectively ($p = 0.002$). The Assessment of Response to Candesartan in Heart Failure in Japan (ARCH-J) study investigated the efficacy of candesartan (8 mg once daily) in comparison with the placebo in 305 patients with symptomatic CHF (Matsumori et al 2003). During the 6-month follow-up period, fatal cardiovascular events occurred in 2 patients in each treatment group and the incidence of progression of CHF was 7% and 22% in the candesartan and the placebo group, respectively ($p = 0.0004$).

We have recently initiated a large outcome study with olmesartan in CHF patients, termed The Supplemental Benefit of Angiotensin II Receptor Blocker in Hypertensive



	Mild-moderate CHF	Moderate-severe CHF
N	316	58
NYHA	II-IV	III-IV
Ejection Fraction (Mean)	≤40% (31.1±6.6%)	≤35% (25.8±5.8%)
Age	66.3±13.3 years	67.2±12.9 years
Male	69.4%	64.4%
Coronary Artery Disease	35.3%	32.2%

Figure 5 Comparison of the prognosis of patients with CHF between Western clinical trials and the CHART-1 study. Copyright © 2007. Reproduced with permission from Shiba N, Takahashi J, Matsuki M. 2007. The CHART Study (Japanese). *Naika*, 99:410–14.
Abbreviations: HYHA, New York Heart Association.

Patients with Stable Heart Failure Using Olmesartan (SUPPORT trial), which is currently the largest outcome study in Japan (Table 3). The purpose of our SUPPORT trial is to examine whether an ARB, olmesartan, in addition to conventional treatment, reduces the mortality and morbidity of hypertensive patients with stable CHF. The primary endpoint is a combined event of all-cause death, nonfatal acute myocardial infarction, nonfatal stroke, and hospital admission due to congestive heart failure. We also aim to evaluate the beneficial effect of olmesartan on the

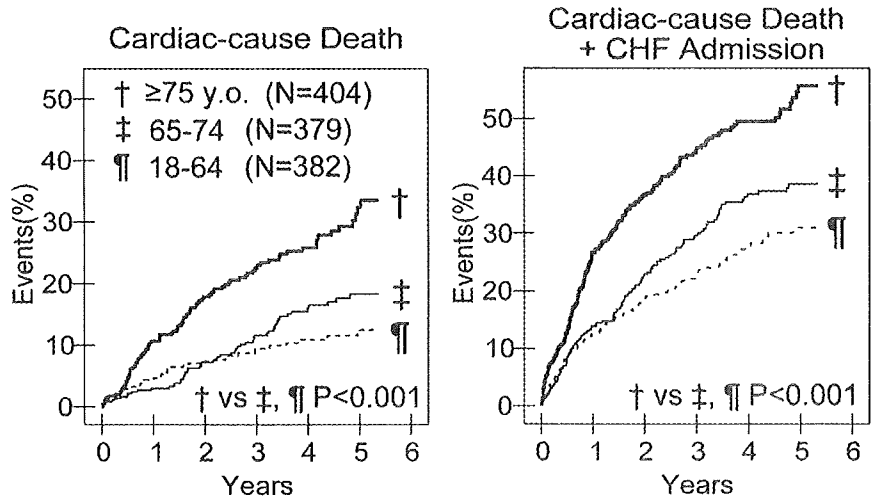


Figure 6 Prognosis of elderly patients with CHF in the CHART-1 study. Copyright © 2007. Reproduced with permission from Shiba N, Takahashi J, Matsuki M. 2007. The CHART Study (Japanese). *Naika*, 99:410–14.
Abbreviations: CHF, congestive heart failure.

progression of metabolic syndrome. The entry of patients was started in November 2007, and the results of the study will be obtained by the end of 2011.

There are 5 other small outcome trials that are currently in progress in Japan (Table 3). The Assessment of Beta-Blocker Treatment in Japanese Patients with Chronic Heart Failure (J-CHF) and the Japanese Diastolic Heart Failure Study (J-DHF) are investigating the effects of carvedilol in patients with systolic CHF and those with diastolic CHF, respectively. Another objective of J-CHF is to determine the optimum dose of carvedilol and to elucidate the differences in clinical characteristics between responders and nonresponders to the β-blocker. The Pitavastatin Heart Failure Study (PEARL study) is designed to evaluate the efficacy of pitavastatin for CHF with mild hypercholesterolemia. The Japanese Multicenter Evaluation of Long- versus short-acting Diuretics in Congestive Heart Failure (J-MELODIC) is designed to compare the effects of furosemide and azosemide in patients with CHF and to test the hypothesis that long-acting diuretics are superior to short-acting ones in those patients. The Japanese Heart Failure Outpatients Disease Management and Cardiac Evaluation Study (J-HOMECARE) was designed to evaluate the benefit of disease management program for prognosis, psychological status and quality of life of patients with CHF.

There are 2 large trials that have investigated the role of valsartan in Japanese patients with cardiovascular disease including CHF (Table 4). The Japanese Investigation of Kinetic Evaluation in Hypertensive Event and Remodeling Treatment (JIKEI-HEART) Study was designed to investigate whether concomitant treatment with valsartan in addition to conventional treatment improves the prognosis of Japanese patients with hypertension, ischemic heart disease, or congestive heart failure. The results of this study have recently been published (Mochizuki et al 2007). After a median follow-up period of 3.1 years, the incidences of stroke, transient ischemic attack, angina pectoris, and CHF were significantly lower in patients treated with valsartan compared with those with the conventional treatment. However, the benefit of the add-on valsartan treatment in a sub-population with CHF, which accounted for 11% of the total population, has not been published yet. The Add-on Effects of Valsartan on Morbi-Mortality (KYOTO-HEART) study was designed to assess the add-on effect of valsartan on the conventional treatment in terms of the morbidity and mortality in Japanese hypertensive patients with high risks of cardiovascular diseases including CHF (Table 4).

Many of the randomized clinical trials performed in Japan utilize the prospective randomized open blinded endpoint (PROBE) design, as an alternative to the randomized double-blind placebo-controlled design (Tables 3 and 4). This is mainly because the PROBE study tends to be more cost effective and its open-labeled medication may minimize ethical considerations (Hansson et al 1992).

Racial difference in morbidity and mortality due to cardiovascular diseases

Several researchers have suggested that cardiovascular risk factors have different prognostic impact among different populations. The Seven Countries Study Research Group showed a substantial heterogeneity among populations in terms of the death rate due to coronary artery disease, even at a similar level of blood pressure (Van den Hoogen et al 2000). The proportion of deaths attributable to cardiovascular disease has also been reported to vary among different cohorts even at the similar serum cholesterol level. The long-term follow-up for 25 years for 12,763 men in 16 cohorts in Europe, the United States, and Japan showed that the risk factors of cardiovascular disease, such as insufficient physical activity and high serum cholesterol level, were not significantly associated with all-cause death in several countries including Japan (Menotti et al 2001). Since these differences cannot be explained by other baseline characteristics, smoking habits, or genetic difference, environmental and/or behavioral factors may play an important role in the development of cardiovascular diseases. The Ni-Hon-San Study, which compared cardiovascular disease rates and risk factors in Japanese men living in Japan, Hawaii, and California, showed that the mortality rate due to coronary artery disease was lowest in Japanese men living in Japan, whereas it was highest in those living in California (Benfante 1992). Racial differences may also influence the effect of medical treatment for CHF. Several studies demonstrated that genetic polymorphisms and/or a difference in β1-receptor sensitivity, which are frequently observed in the Japanese population, might change the pharmacokinetics or the clinical effect of medical drugs, such as ACEI and β-blockers (Kubota et al 2000; Xie et al 2001; Ranade et al 2002).

Future direction for the management of CHF in Japan

CHF is a slowly progressive disease from stage A to stage D unless appropriately treated as described in the ACC/AHA guidelines (Hunt et al 2001). The strategy to manage CHF has been changing recently from treatment to prevention

Table 3 Multicenter randomized clinlical trials for patients with chronic heart failure in Japan

Trial [Reference]	Design	Age, years (Mean)	Comparison	Total enrollment	HF stage NYHA	Study start Expected completion	Mean follow-up	Primary outcome *Published main outcome	Status
MUCHA Trial [Hori 2004]	RDBPC	20–79 (60)	Carvedilol 5 mg Carvedilol 20 mg Placebo	174	C-D II-III		24–48 weeks	Dose-related improvement of HF with carvedilol*	Published
ARCH-J [Matsumori 2003]	RDBPC	≥20 (64)	Candesartan Placebo	305	C-D II-III		6 months	Slowing progression of HF with candesartan*	Published
J-CHF	PROBE	20–79	Carvedilol; 2.5 mg, 5 mg, or 20 mg	480 (exp.)	C-D II-III	Jul 2003 Dec 2009		Cardiovascular mortality Hospitalization for HF	Recruiting
J-DHF	PROBE	≥20	Carvedilol Control	800 (exp.)	C-D	May 2004 Mar 2011		Cardiovascular mortality Hospitalization for HF	Recruiting
SUPPORT Trial	PROBE	20–79	Olmesartan Standard therapy	1000 (exp.)	C-D II-IV	Nov 2006 Sep 2011		Combination of mortality/AMI/Stroke/admission due to HF	Recruiting
PEARL Study	RO	20–79	Pitavastatin Control	500 (exp.)	C-D II-III	Jul 2006 Jul 2010		Cardiac mortality Hospitalization for HF	Recruiting
J-MELODIC	PROBE	≥20	Furosemide Azosemide	300 (exp.)	C-D II-III	Jun 2006 Mar 2010		Cardiovascular mortality Hospitalization for HF	Recruiting
J-HOMECARE	RO	N/A	Education/counseling Control	300 (exp.)	C-D	Dec 2006 Dec 2008		Mortality Readmission for HF	Recruiting

Data are retrieved from published papers or the UMIN Clinical Trials Registry (http://www.umin.ac.jp/ctr/index-j.htm)/ClinicalTrials.gov (http://clinicaltrials.gov/).
Abbreviations: RDBPC, randomized double-blind placebo-controlled design; NYHA, New York Heart Association; HF, heart failure; PROBE, prospective randomized open blinded end-point design; exp., expected; AMI, acute myocardial infarction, RO; randomized open-label design.

Table 4 Multicenter randomized clinlical trials for patients with cardiovascular risks including CHF in Japan

Trial [Reference]	Design	Study population	Age, years (Mean)	Comparison	Total enrollment	Study start Expected completion	Duration	Primary outcome *Published main outcome	Status
JIKEI-Heart Study [Mochizuki 2007]	PROBE	Hypertension coronary disease heart failure	20–79 (65)	Valsartan Standard therapy	3081		3.1 years	Prevention of cardiovascular events with additional valsartan*	Published
KYOTO-HEART Study	PROBE	Hypertension with one or more risk factors including heart failure	20–79	Valsartan Standard therapy	3000 (exp.)	Jan 2004 Oct 2007		Combination of stroke/AMI/CHF etc.	Recruiting

Data are retrieved from published papers or the UMIN Clinical Trials Registry (http://www.umin.ac.jp/ctr/index-j.htm) / ClinicalTrials.gov (http://clinicaltrials.gov/).
Abbreviations: PROBE, prospective randomized open blinded end-point design; exp., expected; AMI, acute myocardial infarction; CHF, congestive heart failure.

(Bansal et al 2006). Japanese CHF patients have several different profiles compared with Western CHF patients as follows; (*a*) the prevalence of CHF of ischemic origin is lower, (*b*) the percentage of elderly population is remarkably high, and (*c*) the penetration rate of evidence-based medicine, such as ACEI/ARB or β-blockers, is not sufficiently high yet. The current situation of the management of CHF in Japan is probably caused by the fact that the number of randomized treatment trials for Japanese patients is not enough yet. Given the expected future increase in Japanese patients with CHF, effective prevention strategy is necessary. Our on-going CHART-2 and SUPPORT studies will enable us to obtain effective strategies to improve the management of CHF in Japan.

Conclusions

The prevalence of CHF will rapidly increase in the next decades in many industrialized countries, including Japan. Large cohort studies with CHF patients are useful for risk stratification and determination of preventive measures for the disorder. Large-scale, randomized treatment trials also are needed, especially in Japan, in order to obtain further evidence to improve the management of patients with CHF.

Acknowledgments

Active investigators of the CHART studies were described in the paper previously published (Shiba et al 2004) and at the website of the Tohoku Heart Failure Association (see http://tohoku.cardiovascular-medicine.jp). The CHART-1 study was supported by the research grants from the Ministry of Health, Labor and Welfare and Gonryo Medical Foundation. We are grateful for the assistance of research nurses who are working at the Tohoku Heart Failure Association; Mika Matsuki, Shizuka Osaki, Kiriko Yukishita, Yuuko Kidoguchi, Miho Hotta, Haruka Kohno, and Keiko Nishiura.

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Clinical Investigation

Incremental Prognostic Values of Serum Tenascin-C Levels With Blood B-type Natriuretic Peptide Testing at Discharge in Patients With Dilated Cardiomyopathy and Decompensated Heart Failure

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ABSTRACT

Background: This study investigates the predictive value of serum tenascin-C (TN-C), which is observed at the active sites of ongoing tissue remodeling, for cardiac events of patients with dilated cardiomyopathy (DCM).

Methods and Results: In this trial, 110 consecutive patients hospitalized with heart failure (HF) resulting from DCM underwent assessments of serum TN-C and plasma brain natriuretic peptide (BNP) levels at discharge and were followed up for 22.4 months. Cardiac function and hemodynamics were assessed invasively in 60 of these patients at discharge. There were 19 cardiac events (14 rehospitalizations, 3 deaths from refractory HF, and 2 sudden deaths) during follow-up. The average levels of TN-C and BNP were 73 ± 38 ng/mL and 279 ± 414 pg/mL, respectively. The optimal cutoff value for serum TN-C levels predicted cardiac events were ≥78.4 ng/mL, whereas BNP levels were ≥219 pg/mL. Patients with levels higher than this had significantly higher cardiac events and serum TN-C ≥78.4 ng/mL had an incremental predictive power with BNP for cardiac events. Left ventricular end-diastolic volume was significantly larger, and mean pulmonary arterial pressure was elevated in patients with serum TN-C ≥78.4 ng/mL.

Conclusions: The combined index of serum levels for TN-C and BNP at discharge predicts cardiac events from decompensated HF. Additionally, elevated serum TN-C levels reflect left ventricular and pulmonary vascular remodeling in DCM patients. (*J Cardiac Fail 2009;■:1–8*)

Keywords: Tenascin-C, B-type natriuretic peptide, dilated cardiomyopathy, prognosis.

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The authors have no conflicts of interest.

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Heart failure (HF) is a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricles to fill with or eject blood. Over time, adverse structural remodeling of the myocardium occurs, including cardiomyocyte hypertrophy, necrosis, and apoptosis, which alter cardiomyocyte mass and cause a progressive accumulation of fibrous tissue.[1–3] The chronically failing heart is characterized by iterations in fibrous tissue formation that will adversely influence tissue stiffness and diastolic mechanics.

Biomarker testing has revolutionized the approach to diagnosis and risk stratification of HF over the past decade. Brain natriuretic peptide (BNP) is augmented primarily by an increase in wall tension in response to pressure (and volume) overload in both the atria and the ventricles. There

is a growing body of consistent literature supporting the utility of blood BNP testing for risk stratification among patients with HF.[4] However, the intraindividual variability of serial BNP levels remains a significant challenge for accurate diagnoses.[5] An enhanced risk assessment would be of great clinical value if it could more accurately identify people who are at increased risk for hospitalization from exacerbated HF.

Tenascin-C (TN-C), an extracellular matrix glycoprotein, is not only expressed during embryogenesis, but also during tissue remodeling.[6,7] In the adult myocardium, TN-C is re-expressed under pathologic conditions such as myocardial infarction,[8,9] myocarditis,[10,11] myocardial hibernation,[12] and dilated cardiomyopathy (DCM).[13–15] Although TN-C molecules are deposited in extracellular spaces, soluble forms of TN-C are also released into the blood stream. In fact, serum TN-C levels in patients with acute myocardial infarction are significantly elevated, reflecting local expression of TN-C in the heart, suggesting the potential use of TN-C as a biomarker to reflect left ventricular (LV) remodeling and to predict prognosis. Furthermore, we previously reported that higher serum TN-C levels reflect the severity of HF, LV dysfunction, and LV remodeling in patients with DCM.[15] It is therefore important to test the possible utility of TN-C as a prognostic biomarker of HF and its incremental prognostic values with blood BNP testing, because BNP and TN-C reflect different disease pathways in assessing the risk of the exacerbated HF.

We hypothesized that serum TN-C at discharge can be used as a biomarker for the risk stratification of HF, and that a combination of 2 biomarkers, TN-C and BNP, could add substantial prognostic information with respect to the risk of the hospitalization and cardiac death from the exacerbated HF in patients with DCM. Accordingly, we investigated the power of serum TN-C levels and blood BNP testing, which were measured at discharge in patients with DCM, as a prognostic biomarker.

Methods

Study Group

A total of 110 patients (79 men, 31 women; mean age 61 ± 14 years) with DCM, who were admitted for HF (New York Heart Association [NYHA] functional Class III to IV) to Osaka Medical College Hospital, Yokosuka Kyosai Hospital, and Mie University Hospital Japan were enrolled in our study. The clinical diagnosis of DCM was made according to the World Health Organization/International Society and Federation of Cardiology task force.[16] Patients were excluded from this study if they suffered lung disease, liver disease, collagen disease, infectious disease, malignancy, end-stage renal disease, or were on hemodialysis. Patients were also excluded from the present study if they had significant coronary stenosis by coronary angiography. After HF symptoms were relieved by optimal therapy including diuretics, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, β-blockers or inotropic agents, serum TN-C, and plasma BNP levels were measured 3 days before discharge. All the patients were followed by the board-certified members of the Japanese Circulation Society at least once per month.

Sixty patients of 110 agreed to undergo cardiac catheterization using conductance catheters with microtip-manometers 3 days before discharge. Right heart catheterization was performed to measure right atrial pressure, pulmonary artery systolic pressure, mean pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac index, and pulmonary vascular resistance. After right heart catheterization and coronary angiography, LV function and hemodynamics were evaluated by conductance catheter (Webster Laboratories, Baldwin Park, CA) with a 2F microtip-manometer (Millar Instruments, Inc, Houston, TX), as described previously.[17] The LV contractile state was assessed by positive dP/dt and LV end-systolic elastance. The time constant of LV relaxation by a non-zero asymptote method, curve-fitting, and stiffness constants, and LV diastolic wall stress were used for assessment of LV diastolic function. The impact of serum TN-C on cardiac function and hemodynamics was evaluated. Written informed consent was obtained from all the patients, and the study was approved by our institutional review boards (ID: 756).

Assay of Serum TN-C Levels by Enzyme-linked Immunosorbent Assay

Blood samples were collected at discharge and were centrifuged at 15,000*g* for 15 minutes, and resulting supernatants were stored at -80°C until analysis. Serum TN-C levels were determined using an enzyme-linked immunosorbent assay kit with 2 monoclonal antibodies, 4F10TT and 19C4MS (IBL, Gunma, Japan), as previously described.[18]

Biochemical Analysis

Plasma BNP concentrations were measured using a specific immunoradiometric commercial assay kit (Shionogi, Japan). Glomerular filtration rate was calculated using the Modification of Diet in Renal Disease Study equation.[19]

End Point

The study end point was a combination of re-hospitalization and cardiac death related to the exacerbation of HF. End point data were collected during follow-up visits.

Statistical Analysis

Statistical analyses were performed using SPSS version 15 (SPSS Inc, Chicago, IL). Continuous data were expressed as mean ± SD and compared using the unpaired Student *t*-test. Categorical data were compared with the chi-square test. Receiver-operating characteristic analysis was used to determine the optimal cutoff value of clinical variables for prediction of cardiac events of rehospitalization and cardiac death from decompensated HF. The best cutoff value was defined as the point with the highest sum of sensitivity and specificity. Event-free survival curves for cardiac events from HF were constructed using the Kaplan-Meier method to visualize the relationship between serum TN-C and BNP at discharge, and statistical differences between curves were assessed using the log-rank test. The effect of clinical predictors on cardiac events was assessed by simple and forward stepwise multivariate Cox proportional hazards models. Multiple linear regression analysis was performed with serum TN-C as the dependent variable. $P < .05$ was considered significant.

Results

In total, 110 DCM patients (79 men, mean age 61 ± 14 years) were enrolled in the present study. At discharge, 38 patients (35%) were in NYHA functional Class III or IV. The average LV ejection fraction measured by 2-dimensional echocardiography was 0.37 ± 0.14, and average values for serum TN-C and plasma BNP at discharge were 73 ± 38 ng/mL and 279 ± 414 pg/mL, respectively.

During the follow-up period (average 22 ± 4 months), there were 19 cardiac events (14 rehospitalizations for worsening HF, 3 deaths from refractory HF, and 2 sudden deaths). There was 1 death of noncardiac origin. The receiver-operating characteristic analysis demonstrated that the optimal cutoff value of serum TN-C at discharge that predicted the combined end point of rehospitalization and cardiac death was ≥78.4 ng/mL, and that of BNP was ≥219 pg/mL (Fig. 1).

Patients with cardiac events (n = 19, 17%) had higher NYHA functional class, larger LV end-diastolic diameter, and higher values for serum TN-C and plasma BNP than those without events (Table 1). As shown in Table 2, patients with serum TN-C ≥78.4 ng/mL at discharge had higher NYHA functional class, larger LV end-diastolic and end-systolic diameters, and higher values for plasma BNP than those with serum TN-C <78.4 ng/mL. There were no significant differences in heart rates, LV ejection fraction, hemoglobin, glomerular filtration rate, total bilirubin, or uric acid between the 2 groups.

Kaplan-Meier Analysis

During the follow-up periods, 14 of 38 patients with BNP ≥219 pg/mL (35%), and 15 patients of 43 with serum TN-C ≥78.4 pg/mL at discharge (28%) had cardiac events of rehospitalization and cardiac death from exacerbated HF. Both BNP ≥219 pg/mL and serum TN-C ≥78.4 pg/mL at discharge had strong prognostic values for HF with DCM ($P < .01$) (Fig. 2).

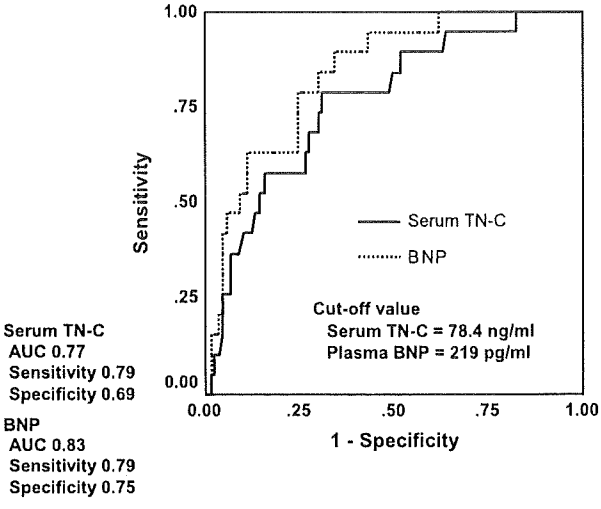

Fig. 1. Receiver-operating characteristic curves for serum tenascin-C (TN-C) and plasma brain natriuretic peptide (BNP) before discharge in 110 dilated cardiomyopathy (DCM) patients for prediction of cardiac events of rehospitalization and cardiac death.

Patients were divided into 4 groups according to their serum TN-C and BNP levels: serum TN-C ≥78.4 ng/mL and BNP ≥219 pg/mL (average serum TN-C 116 ± 33 ng/mL and BNP 757 ± 634 pg/mL, n = 24), serum TN-C ≥78.4 ng/mL and BNP <219 pg/mL (average serum TN-C 103 ± 24 ng/mL and BNP 106 ± 57 pg/mL, n = 19), serum TN-C <78.4 ng/mL and BNP ≥219 pg/mL (average serum TN-C 53 ± 15 ng/mL and BNP 469 ± 219 pg/mL, n = 14) and serum TN-C <78.4 ng/mL and BNP <219 pg/mL (average serum TN-C 47 ± 16 ng/mL and BNP 81 ± 61 pg/mL, n = 53). In the group of serum TN-C ≥78.4 ng/mL and BNP ≥219 pg/mL, 12 patients of 24 were rehospitalized and 2 patients of 12 died from refractory HF (Fig. 3). Kaplan-Meier analysis showed an increased cardiac event rate in this group ($P < .05$) compared with those of other groups (2 rehospitalizations [4%] of 53 in serum TN-C <78.4 ng/mL and BNP < 219 pg/mL, 2 [1 rehospitalization and 1 sudden death] events [14%] of 14 in serum TN-C < 78.4 ng/mL and BNP ≥219 pg/mL, and 3 [1 rehospitalization, 1 death from refractory HF and 1 sudden death] events (16%) of 19 in serum TN-C ≥78.4 ng/mL and BNP <219 pg/mL). There was no difference in cardiac event rate among the other 3 groups (Fig. 3).

Multivariate Predictors for Rehospitalization

The results of simple and multiple Cox proportional hazards models related to cardiac events are shown in Table 3. In simple Cox regression analysis, serum TN-C ≥78.4 ng/mL at discharge was one of the most powerful predictors of cardiac events. The other predictors of cardiac events were renal insufficiency defined as glomerular filtration rate less than 60 mL·min·1.73 m^2, total bilirubin ≥1.2 mg/dL, NYHA functional Class III/IV, and plasma BNP ≥219 pg/mL. The stepwise multivariate analysis showed that serum TN-C ≥78.4 ng/mL ($P = .002$), renal insufficiency ($P = .002$), high total bilirubin ($P = .025$), and BNP ($P = .022$) were the independent predictors of cardiac events from exacerbated HF.

Multiple Linear Regression Analysis Evaluating Serum TN-C and Clinical Variables

In multiple linear regression analysis, serum TN-C levels were independent of the clinical variables of age, gender, body mass index, hemoglobin, glomerular filtration rate, uric acid, total bilirubin, and the use of angiotensin-converting enzyme inhibitors and/or angiotensin II receptor blockers, β-blockers, or diuretics.

Comparison of Cardiac Function and Hemodynamics

Results of heart catheterizations in the subset of 60 patients were compared between the serum TN-C ≥78.4 ng/mL group and <78.4 ng/mL group. LV end-diastolic

Table 1. Patient Characteristics at Discharge

Variables	All Patients (n = 110)	Events (-) (n = 91)	Events (+) (n = 19)	*P*
Age (y)	61 ± 14	60 ± 13	64 ± 17	.34
Male gender, n (%)	79 (72)	67 (74)	12 (63)	.36
Body mass index (kg/m^2)	22.2 ± 3.3	22.6 ± 3.4	19.8 ± 1.8	<.001
NYHA functional Class III/IV, n (%)	38 (34)	25 (28)	13(68)	.001
Heart rates (beats/min)	74 ± 13	73 ± 13	75 ± 14	.69
Systolic blood pressure (mm Hg)	118 ± 23	120 ± 23	109 ± 19	.029
Diastolic blood pressure (mm Hg)	70 ± 13	72 ± 13	62 ± 13	.003
Comorbidity, n (%)				
Hypertension	41 (37)	39 (43)	2 (11)	.008
Diabetes mellitus	29 (27)	25 (27)	4 (21)	.57
Atrial fibrillation	35 (32)	27 (30)	8 (42)	.29
Medications, n (%)				
ACEI or ARB	95 (86)	78 (86)	17 (90)	.67
β-blockers	52 (47)	45 (49)	7 (37)	.32
Diuretics	78 (71)	59 (65)	19 (100)	.002
Echocardiography				
LV end-diastolic diameter (mm)	61 ± 9	60 ± 9	65 ± 9	.048
LV end-systolic diameter (mm)	50 ± 11	49 ± 10	54 ± 10	.052
LV ejection fraction (%)	37 ± 14	38 ± 14	32 ± 12	.088
Left atrial diameter (mm)	44 ± 8	44 ± 8	46 ± 8	.41
Laboratory measurements				
Serum hemoglobin (g/dL)	13.4 ± 1.8	13.7 ± 1.7	12.0 ± 1.7	<.001
Estimated GFR (mL·min·1.73 m^2)	75 ± 33	77 ± 33	66 ± 23	.12
Total bilirubin (mg/dL)	0.9 ± 0.8	0.7 ± 0.4	1.4 ± 1.7	.14
Uric acid (mg/dL)	6.6 ± 1.7	6.6 ± 1.7	6.1 ± 2.0	.31
Serum TN-C (ng/mL)	73 ± 38	67 ± 36	100 ± 37	.002
BNP (pg/mL)	279 ± 414	216 ± 380	579 ± 450	.003
C-reactive protein (mg/L)	0.3 ± 0.4	0.2 ± 0.3	0.4 ± 0.4	.22

NYHA, New York Heart Association; LV, left ventricular; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; GFR, glomerular filtration rate; TN-C, tenascin-C; BNP, brain natriuretic peptide.

Values are expressed as mean ± SD or n (%).

P values are for patients with events versus those without events and $P < .05$ was significant.

(214 ± 74 vs. 178 ± 43 mL, $P = .047$) and end-systolic volume (173 ± 73 vs. 136 ± 47 mL, $P = .043$) were significantly larger in the high TN-C group than low TN-C group without changes in heart rate. There were no significant differences in pulmonary capillary wedge pressure (16 ± 10 vs. 12 ± 7 mm Hg) and right atrial pressure (5 ± 3 vs. 4 ± 3 mm Hg) between the 2 groups. Systolic and mean pulmonary arterial pressures (25 ± 11 vs. 18 ± 7 mm Hg, $P < .01$) were elevated in the high TN-C group compared with the low TN-C group, indicating ongoing pulmonary vascular remodeling in the high TN-C group. There were no significant differences in LV systolic function assessed by positive dP/dt, LV ejection fraction, and LV end-systolic elastance (0.8 ± 0.5 vs. 0.9 ± 0.4 mm Hg/mL). There were also no significant differences in LV diastolic function assessed by the time constant of LV relaxation (51 ± 13 vs. 51 ± 10 ms), curve-fitting (1.5 ± 2.0 vs. 1.9 ± 3.7) and stiffness constants (0.020 ± 0.023 vs. 0.023 ± 0.017), or LV diastolic wall stress (48 ± 38 vs. 34 ± 19 kdyne/cm^2) between the 2 groups. When results of heart catheterizations were compared between the plasma BNP ≥219 pg/mL group and <219 pg/mL group, systolic parameters assessed by LV end-systolic elastance (0.5 ± 0.3 vs. 1.0 ± 0.5 mm Hg/mL) and LV ejection fraction (24 ± 8 vs. 35 ± 10%) were significantly lower and pulmonary capillary wedge pressure (18 ± 9 vs. 10 ± 6 mm Hg, $P < .01$) was higher in high BNP group than low BNP group, in addition to larger LV end-diastolic and end-systolic volume and higher systolic and mean pulmonary arterial pressures. There were also no significant differences in LV diastolic function assessed by the time constant of LV relaxation, and stiffness constants, or LV diastolic wall stress between the 2 groups. Thus, an increased plasma BNP may reflect LV pump failure, whereas an increased serum TN-C may reflect ongoing LV or pulmonary remodeling.

Discussion

In the present study, we demonstrated that serum TN-C levels ≥78.4 ng/mL at discharge were associated with the adverse outcomes of rehospitalization and cardiac death due to exacerbated HF. Furthermore, combined serum TN-C levels with plasma BNP levels at discharge were a stronger predictor of cardiac events for HF than either single biomarker alone.[20–22] These findings support the rationale for adding multiple biomarkers that reflect different disease pathways in assessing the risk of decompensated HF in patients with DCM.

TN-C is not detected in the normal adult myocardium, but appears under pathophysiologic conditions during tissue remodeling.[8,10,23] TN-C loosen the attachment of cardiomyocytes to connective tissues[8] and upregulates expression and activity of matrix metalloproteinases,[24] which may promote degradation of connective tissue and contribute to LV dilation. Furthermore, tenascin-C could be also a key

Table 2. Patient Characteristics at Discharge

Variables	Serum TN-C (ng/mL) ≥78.4 (n = 43)	< 78.4 (n = 67)	*P* Value
Age (y)	59 ± 16	62 ± 13	.42
Male gender, n (%)	29 (67)	50 (75)	.52
Body mass index (kg/m^2)	21.6 ± 3.0	22.6 ± 3.6	.12
NYHA functional Class III/IV, n (%)	26 (60)	12 (18)	<.001
Heart rates (beats/min)	76 ± 15	72 ± 12	.10
Systolic blood pressure (mm Hg)	116 ± 29	119 ± 18	.52
Diastolic blood pressure (mm Hg)	67 ± 12	72 ± 14	.052
Comorbidity, n (%)			
Hypertension	12 (28)	29 (43)	.11
Diabetes mellitus	9 (21)	20 (30)	.38
Atrial fibrillation	19 (44)	16 (24)	.026
Medications, n (%)			
ACEI or ARB	39 (91)	56 (84)	.56
β-blockers	15 (35)	37 (55)	.038
Diuretics	34 (79)	44 (66)	.13
Echocardiography			
LV end-diastolic diameter (mm)	63 ± 9	60 ± 8	.036
LV end-systolic diameter (mm)	53 ± 11	48 ± 10	.008
LV ejection fraction (%)	33 ± 13	39 ± 14	.063
Left atrial diameter (mm)	47 ± 8	43 ± 8	.16
Laboratory measurements			
Serum hemoglobin (g/dL)	13.1 ± 2.0	13.6 ± 1.6	.15
Estimated GFR (mL·min·1.73 m^2)	73 ± 28	77 ± 36	.61
Total bilirubin (mg/dL)	1.1 ± 1.3	0.7 ± 0.4	.090
Uric acid (mg/dL)	6.7 ± 1.8	6.4 ± 1.7	.61
Serum TN-C (ng/mL)	110 ± 30	48 ± 16	<.001
BNP (pg/mL)	469 ± 573	156 ± 189	.001
C-reactive protein (mg/L)	0.4 ± 0.5	0.2 ± 0.2	.009

NYHA, New York Heart Association; LV, left ventricular; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; GFR, glomerular filtration rate; TN-C, tenascin-C; BNP, brain natriuretic peptide.

Values are expressed as mean ± SD or n (%).

P values are for TN-C ≥78.4 ng/mL versus TN-C < 78.4 ng/mL and *P* < .05 was significant.

regulator in early step of fibrosis, and its elevation may suggest active progression of the fibrosis.[10,25–28] It is known that ventricular remodeling is accompanied by changes of the extracellular matrix, and that several molecules related matrix turnover such as matrix metalloproteinases, and their tissue inhibitors, carboxy-terminal telopeptide of type I collagen, amino-terminal propeptide of type I and III procollagen have received much attention as significant biomarkers in HF. TN-C may be a novel candidate for marker of extracellular matrix remodeling.

The expression of TN-C has been reported in the myocardium of DCM patients[13] and in the pulmonary arteries of animals with hypertensive remodeling.[29] Patients with familial pulmonary arterial hypertension also have higher serum TN-C, which promotes the proliferation of both pulmonary arterial smooth muscle cells and endothelial cells.[30] Our hemodynamic measurements showed that patients with serum TN-C ≥78.4 ng/mL had elevated pulmonary arterial pressures and resistance compared with those with serum TN-C <78.4 ng/mL, whereas there were no differences in the values for pulmonary capillary wedge pressure or LV diastolic wall stress between the 2 groups. These findings suggest that TN-C molecules might be produced not only from the remodeling left ventricles but also from the pulmonary vascular beds. Hörstrup et al reported that urinary tenascin levels were elevated in patients with nephropathies; however, serum TN-C concentrations did not increase with progressive reduction in renal function.[31] In the present study, there was no difference in glomerular filtration rate between the 2 groups and multiple linear regression analysis also showed that serum TN-C levels were independent of glomerular filtration rate.

Current medical management of patients with HF relies on patients' and physicians' subjective clinical assessment and various nonspecific laboratory measurements of organ dysfunction and fluid status. HF progresses through a process of structural remodeling of the heart, to which neurohormonal activation, including BNP, make important contributions. Blood BNP testing is valuable for prognosis and risk stratification. Cardiac events correlate with BNP levels at discharge and during outpatient follow-up. Dokainish et al found that discharge BNP levels <250

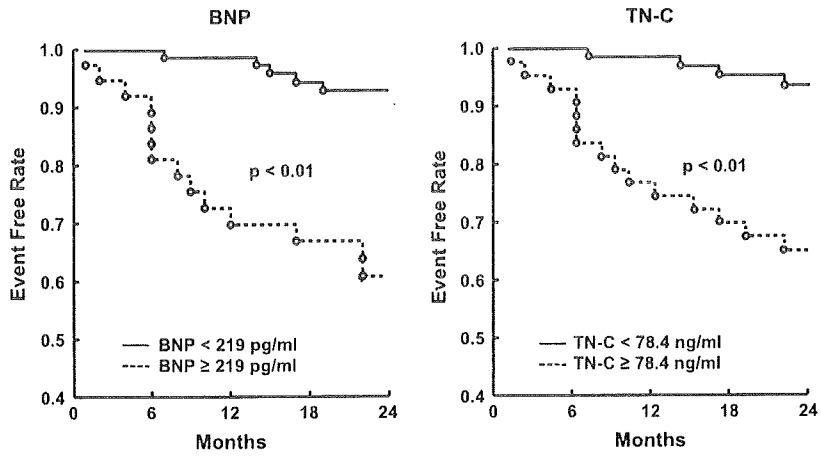

Fig. 2. Kaplan-Meier analysis showing higher cardiac events rate in dilated cardiomyopathy (DCM) patients with their brain natriuretic peptide (BNP) ≥219 pg/mL and in those with their serum tenascin-C (TN-C) ≥78.4 ng/mL at discharge.

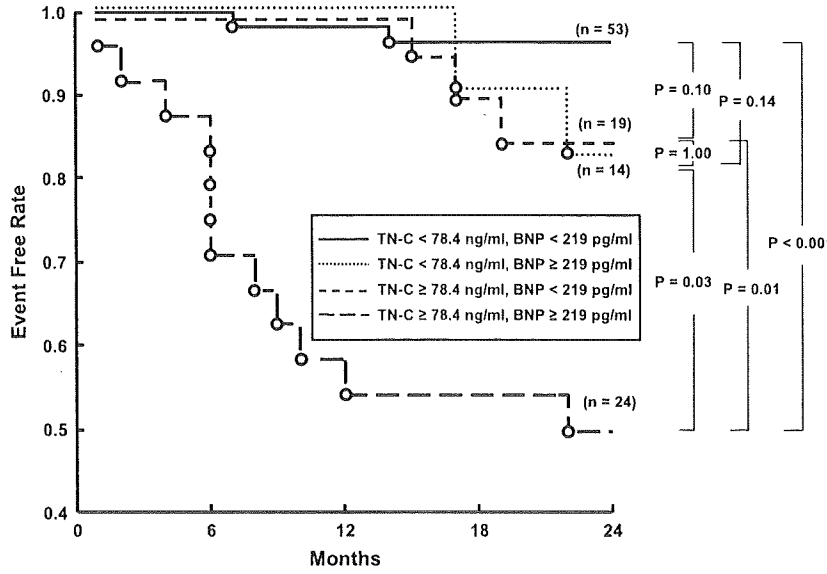

Fig. 3. Kaplan-Meier analysis of cardiac events-free survival for the four risk groups of 110 dilated cardiomyopathy (DCM) patients based on serum tenascin-C (TN-C) and plasma brain natriuretic peptide (BNP) concentrations.

pg/mL predicted event-free survival.[21] Nishii et al reported that a 6-month postdischarge BNP measurement of >190 pg/mL predicted a long-term risk of redecompensation in stable low-risk outpatients with DCM after decompensated HF.[32] Similarly to these previous reports, we showed that plasma BNP ≥219 pg/mL was a predictor of rehospitalization and cardiac death from exacerbated HF. However, BNP levels fall rapidly after diuretic therapy in patients with decompensated HF, although these changes may vary widely and can be independent of hemodynamic changes. The intraindividual variability of serial BNP levels remains highly debated.[5] It is noteworthy that, in the present study, the combination of BNP and TN-C predicted cardiac events for decompensated HF to a greater extent than either biomarker individually. BNP is secreted from cardiomyocytes in response to increasing cardiac wall tension, whereas TN-C is synthesized in interstitial fibroblasts that were provoked by various proinflammatory cytokines, hypoxia, acidosis, and mechanical stress.[8,10] We cannot neglect the effect of cardiac fibroblasts on LV remodeling because they are the most numerous cells in the heart. Thus, BNP and TN-C make distinct contributions to LV remodeling so that the combination of serum TN-C with plasma BNP may have a synergistic effect to stratify patients at very high risk for adverse cardiac events of rehospitalization and cardiac death from exacerbated HF.

We also showed that the other predictor of cardiac events independent of these biomarkers was renal dysfunction. Renal dysfunction is a common complication of chronic HF, with a clinical course that typically fluctuates with the patient's clinical status and treatment. In ambulatory HF patients, the presence of concomitant renal dysfunction has been one of the most consistent risk factors for mortality. This risk becomes evident even at serum creatinine levels >1.3 mg/dL and estimated creatinine clearance values ≤60 to 70 mL/min.[22,33] Although renal dysfunction

Table 3. Predictors of Cardiac Events in Cox Proportional Hazards Models

Variables	Number of Patients with Variables (%)	Simple Model		Multivariate Model	
		P Value	Risk Ratio (95% CI)	*P* Value	Risk Ratio (95% CI)
Age ≥65 y	54 (49)	.45	1.42 (0.57-3.53)	.58	
Female gender	31 (28)	.35	1.56 (0.61-3.96)	.27	
Anemia, hemoglobin <12.0 g/dL in women <13.0 g/mL in men	33 (30)	.16	1.92 (0.77-4.78)	.21	
Renal insufficiency, GFR < 60 mL·min·1.73 m^2	33 (30)	.002	4.30 (1.73-10.73)	.002	4.68 (1.79-12.24)
Hyperuricemia, uric acid ≥7.0 mg/dL	46 (42)	.95	1.03 (0.41-2.56)	.70	
Jaundice, total bilirubin ≥1.2 mg/dL	12 (11)	.024	3.24 (1.16-8.99)	.025	3.59 (1.17-11.02)
BNP ≥219 pg/mL	38 (35)	.004	4.02 (1.58-10.21)	.022	3.42 (1.19-9.85)
Serum TN-C ≥78.4 ng/mL	43 (39)	.001	6.70 (2.22-20.21)	.002	6.52 (1.97-21.55)
NYHA Class III/IV	38 (34)	.001	5.53 (2.10-14.59)	.39	
β-blocker use	52 (47)	.37	0.65 (0.26-1.66)	.42	

GFR, glomerular filtration rate; NYHA, New York Heart Association; BNP, brain natriuretic peptide; TN-C, tenascin-C.