Elevated Levels of Both Cardiomyocyte Membrane and Myofibril Damage Markers Predict Adverse Outcomes in Patients With Chronic Heart Failure

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Background Recent studies have shown the presence of ongoing myocardial damage in patients with chronic heart failure (CHF) detected by myofibril and membrane damage markers, cardiac troponin T (TnT) and heart-type fatty acid-binding protein (H-FABP), which identifies patients at increased risk of a future cardiac event (CE: death or rehospitalization because of worsening CHF). There is a difference between TnT and H-FABP in their release kinetics following myocardial damage.

Methods and Results TnT and H-FABP were measured in 103 patients with CHF and in 31 controls. Patients were classified into 4 groups based on detectable (≥ 0.01 ng/ml) or undetectable TnT (TnT+ or TnT-) and H-FABP $\geq$ or < 4.5 ng/ml (mean ± 2 standard deviations in controls) (high-H-FABP or low-H-FABP). Kaplan-Meier analysis showed that the CE-free rate ($n=43$) was significantly lower in patients with TnT+ and high-H-FABP than in patients in the other 3 groups (patients with TnT+ and low-H-FABP, TnT- and high-H-FABP, and TnT- and low-H-FABP; $p=0.02$, $p=0.001$ and $p=0.0002$, respectively). In stepwise multivariate Cox proportional hazard analysis, TnT+ ($p=0.01$) and high-H-FABP ($p=0.04$) were independent predictors of future CE.

Conclusions Elevated levels of both TnT and H-FABP predict adverse outcomes in CHF patients. (Circ J 2008; 72: 569–574)

Key Words: Biomarkers; Heart failure; Prognosis

Heart failure (HF) is a global health problem. Patients with HF have high readmission rates after discharge¹ and it is therefore important to be able to assess their prognosis to prevent readmission. There are several prognostic indicators of HF. Ventricular remodeling is the major mechanism of progression and is closely related to prognosis.^{2,3} Cardiomyocyte loss resulting from myocardial damage and fibrosis principally contribute to ventricular remodeling.^{4,5} Recent studies have reported the clinical significance of ongoing myocardial damage in patients with chronic HF (CHF) detected by cardiac troponin T (TnT) or I⁶ for identifying patients at increased risk of a subsequent cardiac event (CE).^{7–12} Serum levels of heart-type fatty acid-binding protein (H-FABP), a low-molecular protein that is abundant in the cytosol of cardiomyocytes and is a useful marker of early phase acute myocardial infarction,^{13,14} are also increased^{9,15,16} and reflect ongoing myocardial damage¹⁷ in patients with CHF. However, there is a difference between TnT and H-FABP in their release

kinetics following myocardial damage and H-FABP measurement compensates for the limitations of TnT in the early phase of acute myocardial infarction.¹⁸ Thus, we reasoned that the combination of both markers would be clinically useful for pathophysiological analysis of ongoing myocardial damage in patients with CHF. We previously reported that both TnT and H-FABP were independent predictors of future CE in patients with CHF,⁷ but there have been no reports concerning the usefulness of combined TnT and H-FABP measurements in patients with CHF. The purpose of the present study was to elucidate whether the combination of TnT, a marker of myofibril damage, and H-FABP, a marker of membrane damage, can add value to the assessment of the severity and prognosis of CHF.

Methods

Study Population

The study population consisted of 103 patients with CHF (left ventricular ejection fraction (LVEF) $< 50\%$; 37 women, 66 men; aged 67 ± 12 years) who were hospitalized from December 1999 to October 2004, and 31 age- and gender-matched controls (13 women, 18 men; aged 65 ± 7 years) visiting the outpatient clinic and who were diagnosed as free of cardiovascular and other disease. The diagnosis of CHF was based on the Framingham criteria.¹⁹ Exclusion criteria were: recent (within 3 months) ischemic heart disease and myocarditis, malignancy, muscle disorders (creatinine kinase (CK) > 250 IU/L in women or > 300 IU/L in men), renal failure (creatinine > 2.0 mg/dl) or active pulmonary, liver, collagen or infectious diseases. The severity of CHF was New York Heart Association (NYHA) class II in 89, class III in

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Table 1 Baseline Characteristics of the 4 Groups of Patients

	All (n=103)	TnT+ High-H-FABP (n=25)	TnT- High-H-FABP (n=27)	TnT+ Low-H-FABP (n=10)	TnT- Low-H-FABP (n=41)
Age, years	67±12	68±11	70±11 [‡]	68±10	64±13
Men	66 (64%)	19 (76%)	16 (59%)	5 (50%)	26 (63%)
NYHA class II/III/IV	89/11/3	15*/‡/7*/3	25/2/0	10/0/0	39/2/0
Etiology					
DCM	41 (40%)	10 (40%)	9 (33%)	2 (20%)	20 (49%)
Hypertensive HD	26 (25%)	5 (20%)	6 (22%)	3 (30%)	12 (29%)
Previous MI	28 (27%)	8 (32%)	11 (41%) [‡]	3 (30%)	6 (15%)
Valvular HD	7 (7%)	2 (8%)	1 (4%)	2 (20%)	2 (5%)
Congenital HD	1 (1%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)
CTR, %	57±8	60±9	56±7	59±7	57±8
Cr, mg/dl	1.0±0.3	1.2±0.4 ^{†,‡}	1.1±0.4 ^{†,‡}	0.8±0.1	0.8±0.2
CK, IU/L	75±61	73±50	72±85	74±64	78±50
CK-MB, IU/L	6±5	9±6 ^{*,†,‡}	5±5	5±4	4±4
H-FABP, ng/ml	5.6±3.4	9.4±4.6 ^{*,†,‡}	6.2±1.3 ^{†,‡}	3.6±0.5	3.3±0.8
ANP, pg/ml	107±103	175±152 ^{*,†,‡}	70±59	91±51	93±80
BNP, pg/ml	349±398	593±581 ^{*,‡}	263±358	316±248	264±230
NE, pg/ml	593±392	725±498	554±308	452±587	573±308
LVEF, %	34±10	30±10 [‡]	33±9	36±10	36±11

**p*<0.05 vs TnT- and high-H-FABP; [†]*p*<0.05 vs TnT+ and low-H-FABP; [‡]*p*<0.05 vs TnT- and low-H-FABP.

TnT+, detectable troponin T; TnT-, undetectable troponin T; H-FABP, heart-type fatty acid-binding protein; NYHA, New York Heart Association; DCM, dilated cardiomyopathy; HD, heart disease; MI, myocardial infarction; CTR, cardiothoracic ratio; Cr, creatinine; CK, creatine kinase; ANP, A-type natriuretic peptide; BNP, B-type natriuretic peptide; NE, norepinephrine; LVEF, left ventricular ejection fraction.

11, and class IV in 3 patients. The etiology of CHF was dilated cardiomyopathy in 41 patients, previous myocardial infarction in 28, hypertensive heart disease in 26, valvular heart disease in 7, and congenital heart disease in 1 patient. Informed consent was given by all participants.

Measurement of Biochemical Markers

Blood samples were obtained from the CHF patients when their condition had stabilized (5–20 days after hospitalization). Serum levels of TnT and H-FABP were measured using an enzyme-linked immunosorbent assay (TnT: Elecsys Troponin T, Roche Diagnostics, Mannheim, Germany; detection limit: 0.01 ng/ml; and H-FABP: MARKIT-M; Dainippon-Sumitomo Pharmaceutical Company, Osaka, Japan; detection limit: 1.25 ng/ml). Plasma levels of A- and B-type natriuretic peptides (ANP and BNP) (ANP: Shionoria ANP, Shionogi Co Ltd, Osaka, Japan and BNP: Shionoria BNP, Shionogi Co Ltd), norepinephrine (HLC-725 CA II, Tosoh Co, Tokyo, Japan) and serum levels of CK (Shikarikid CK, Kanto Medical Co, Inc, Tokyo, Japan) and CK-MB (Shikarikid CK-MB, Kanto Medical) were measured simultaneously. Other clinical parameters included the cardiothoracic ratio calculated from chest radiograph and LVEF evaluated by ^{99m}technetium albumin radionuclide ventriculography. Chest radiography and radionuclide ventriculography were performed within 2 weeks of the measurement of TnT and H-FABP.

Classification of Patients

Patients with CHF were classified into 4 groups: (1) detectable TnT (≥0.01 ng/ml; TnT+) and H-FABP ≥4.5 ng/ml (mean+2 standard deviations in controls; high-H-FABP), (2) TnT+ and H-FABP <4.5 ng/ml (low-H-FABP), (3) undetectable TnT (TnT-) and high-H-FABP, and (4) TnT- and low-H-FABP.

Follow-up

All patients were followed up for 28±26 months. The

endpoint was death or rehospitalization because of worsening CHF (ie, CE).

Statistical Analysis

Results are expressed as mean ± standard deviation. Comparisons of clinical parameters between the patients with CHF and the controls were performed using an unpaired t-test. Clinical characteristics among the 3 groups were compared by an analysis of variance with the Fisher post hoc test. TnT detection ratios were compared with the chi-square test. CE-free curves were calculated using the Kaplan-Meier method. The association between gender, TnT+, high-H-FABP, NYHA class, age, creatinine, BNP and norepinephrine > median value and LVEF < median value and CE were tested in univariate Cox regression models (NYHA class, creatinine, BNP, norepinephrine and LVEF are established prognostic predictors of CHF). Stepwise multivariate Cox proportional hazard analyses were performed using the same parameters as for the univariate analyses and the same parameters plus the combination of TnT+ and high-H-FABP. A *p*-value <0.05 was considered statistically significant.

Results

There were no significant differences between controls and patients with CHF in age, gender and serum levels of CK and CK-MB at baseline. The TnT detection ratio and H-FABP levels in patients with CHF were higher than in the controls (TnT detection ratio: 35/103 vs 0/31, *p*=0.0004, H-FABP: 5.6±3.4 vs 2.9±0.8 ng/ml, *p*<0.0001).

Table 1 shows the clinical characteristics of the patients in the 4 groups. There were no significant differences among them in gender, CK, norepinephrine or cardiothoracic ratio. CK-MB and ANP levels were significantly higher in the group of patients with TnT+ and high-H-FABP than in the other 3 groups. BNP was significantly higher in patients with TnT+ and high-H-FABP than in those with TnT+ and

Table 2 Medications

	All (n=103)	TnT+ High-H-FABP (n=25)	TnT- High-H-FABP (n=27)	TnT+ Low-H-FABP (n=10)	TnT- Low-H-FABP (n=41)
Diuretics	90 (87%)	24 (96%)	25 (93%)	8 (80%)	33 (80%)
Aldosterone blockers	29 (28%)	6 (24%)	6 (22%)	1 (10%)	16 (39%)
ACEI or ARB	80 (78%)	20 (80%)	21 (78%)	7 (70%)	32 (78%)
Digitalis -blockers	57 (55%)	15 (60%)	12 (44%)	6 (60%)	24 (59%)
	30 (29%)	9 (36%)	7 (26%)	3 (30%)	11 (27%)

ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers. Other abbreviations as in Table 1.

Fig 1. Cardiac event-free survival curves in patients with chronic heart failure (CHF) (A) and CHF patients without previous myocardial infarction (B). TnT(+), detectable troponin T; TnT(-), undetectable troponin T; H-FABP, heart-type fatty acid-binding protein.

low-H-FABP, and with TnT- and low-H-FABP. There were no significant differences in the medical treatments of the 4 groups (Table 2).

Kaplan-Meier cumulative CE-free curves in each group are shown in Fig 1A. Over a mean follow-up period of 28 ± 26 months, 15 patients died (13 patients died of worsening HF during hospitalization, but according to electrocardiography and biochemical examinations their deaths were not related to ischemic events; 2 patients had sudden cardiac death associated with worsening HF. In 4 of the 15 patients,

the etiology of CHF was a previous myocardial infarction) and 28 patients were rehospitalized because of worsening HF. The cumulative CE-free rate estimated by Kaplan-Meier curves was significantly lower in patients with TnT+ and high-H-FABP compared with that of the patients in the other 3 groups (26% vs 78% in TnT- and low-H-FABP, 70% in TnT- and high-H-FABP, and 90% in TnT+ and low-H-FABP at 2 years, $p=0.0002$, 0.001 and 0.02 , respectively). Fig 1B shows the Kaplan-Meier cumulative CE-free curves in each group of CHF patients without previous myo-

Table 3 Univariate Analyses and Stepwise Multivariate Analyses

	Univariate analyses		Stepwise multivariate analyses			
	HR (95%CI)	p value	HR (95%CI)	p value	HR (95%CI)	p value
Age >68 years	1.24 (0.68–2.25)	0.49		0.60		0.22
Male gender	1.24 (0.70–2.59)	0.37		0.69		0.83
NYHA class III or IV	2.57 (1.23–5.38)	0.01		0.14		0.99
TnT+	2.57 (1.41–4.69)	0.002	2.24 (1.21–4.14)	0.01		0.83
High-H-FABP	2.28 (1.21–4.29)	0.01	1.95 (1.02–3.71)	0.04		0.67
BNP >217.4 pg/ml	2.04 (1.10–3.76)	0.02		0.17		0.39
NE >490 pg/ml	1.44 (0.79–2.64)	0.24		0.05		0.11
LVEF <34.8%	2.03 (1.08–3.80)	0.03		0.09		0.10
Cr >0.9 mg/dl	1.26 (0.68–2.32)	0.46		0.72		0.69
TnT+, high-H-FABP	4.03 (2.19–7.41)	<0.0001	– (–)	–	3.82 (2.04–7.15)	<0.0001

HR, hazard ratio; CI, confidence interval. Other abbreviations as in Table 1.

cardial infarction. The cumulative CE-free rate estimated by the Kaplan-Meier curves was also significantly lower in patients with TnT+ and high-H-FABP compared with that of patients in the other 3 groups (34% vs 84% in TnT– and low-H-FABP, 74% in TnT– and high-H-FABP, and 100% in TnT+ and low-H-FABP at 2 years, $p=0.002$, 0.03 and 0.04, respectively).

Univariate analyses showed that NYHA class III or IV, TnT+, high-H-FABP, BNP >217.4 pg/ml, LVEF <34.8% and TnT+ and high-H-FABP were significant predictors of subsequent CE. Stepwise multivariate Cox proportional hazard analyses revealed that of the 9 variables TnT+ and high-H-FABP were significant and independent predictors of future CE, and only the combination of TnT+ and high-H-FABP was a significant and independent predictor of CE among the 9 variables plus the combination of TnT+ and high-H-FABP (Table 3).

Discussion

Differences in TnT and H-FABP, Myocardial Damage Markers

TnT is a sensitive and specific marker of minor myocardial damage. It is primarily a component of myofibrils;²⁰ thus elevated levels are suggestive of myofibril damage. TnT is released into the circulating blood for several days after the onset of acute myocardial infarction,²⁰ which suggests that TnT exists in the circulation for several days following the leakage of cardiomyocyte contents. Therefore, increased TnT levels are indicative of not only current but also recent myofibril damage.

H-FABP is abundant in the cytosol and is readily released into the circulation following myocardial membrane damage.²¹ Its release kinetics are different from those of TnT. In patients with acute myocardial infarction, serum levels of H-FABP return to normal by 24 h after the onset of myocardial infarction;²² which suggests that H-FABP is rapidly cleared from circulation following leakage from the cytosol. Therefore, H-FABP levels reflect the current extent of myocardial membrane damage.

TnT and H-FABP Levels in Patients With CHF

The present study showed that elevated levels of both TnT and H-FABP reflected severity and a poor prognosis in patients with CHF. The increased levels of both markers are suggested to be the result of existing myofibril and membrane damage of cardiomyocytes. Studies have shown that leakage of cytosolic enzymes occurs during the early stage of anoxia, but is related to reversible membrane altera-

tions;²³ they also suggest that the degradation of the troponin subunits occurs 4 h after coronary artery occlusion.²⁴ So, although it is possible to recover from cardiomyocyte membrane injury, myofibril damage might be irreversible and it is likely that the increase in both markers identifies continuous irreversible cardiomyocyte damage in CHF patients.

In the present study, H-FABP increased but TnT was not detectable in 27 patients; on the other hand, TnT was detectable without a H-FABP increase in 10 patients. It is assumed that patients with increased H-FABP but undetectable TnT have myocardial membrane damage but no obvious myofibril damage. Thus, myocardial damage in patients with increased H-FABP only is probably less severe than that in patients with increased levels of both TnT and H-FABP. In fact, the present study showed that ANP and BNP levels and the CE rates were significantly higher in patients with increased levels of both markers than in patients with increased H-FABP levels only.

What about myocardial damage in patients with detectable TnT but non-increasing H-FABP? H-FABP is readily released into the circulation following myocardial membrane damage but in cases of acute myocardial infarction the circulating level soon returns to the normal range following leakage of cardiomyocyte contents.²² In contrast, the time from the occurrence of myocardial damage to an increased circulating level and that from leakage of cardiomyocyte contents to the return to the normal circulating level is longer for TnT than for H-FABP. Therefore, it is thought that patients with TnT+ but low-H-FABP no longer have current myocardial membrane damage but do have recent myofibril damage. The time-course of ongoing myocardial damage in CHF is still unclear, but our previous report showed that in most patients with CHF, TnT decreased after medical treatment.⁷ Del Carlo et al reported that CHF patients with detectable TnT within 4 days of admission, but undetectable TnT 7 days later or undetectable TnT on both days, had lower rates of death, rehospitalization or both than patients with detectable TnT on both days or detectable TnT at the time of the second measurement.²⁵ Sato et al showed that the CE-free rate was significantly lower in patients with continuous TnT detection than in patients in whom TnT became undetectable or was continuously undetectable.⁸ Thus, the prognosis in patients with TnT that becomes undetectable is better than that for patients with continuously detectable TnT. The present study showed that the CE rate and ANP levels were lower in patients with detectable TnT but non-increasing H-FABP than in patients with increased levels of both markers. However, BNP levels in the first group of patients were not significantly

lower than those seen in patients with increased levels of both markers. The number of patients with TnT+ and low-H-FABP is small, so there were no significant differences in BNP levels between the 2 groups.

It is suggested that CHF patients with elevated levels of both TnT and H-FABP have more severe myocardial damage than patients who only have elevated TnT or H-FABP and that this reflects a poor prognosis. There are few reports concerning the risk stratification of patients with CHF using these 2 biomarkers. Ishii et al proposed that measuring the combination of TnT and BNP concentrations at admission may be a highly effective means of risk stratification of patients with CHF.¹⁰ Niizeki et al reported that the combined measurement of H-FABP and BNP might be a highly reliable evaluation for risk stratification of CHF.²⁶ BNP is an established prognostic predictor in patients with CHF. We assume that the reason why BNP was not an independent prognostic predictor in the present study is the significant correlation between BNP and H-FABP levels ($r=0.41$, $p<0.0001$). We previously reported a significant correlation between BNP and H-FABP levels, and H-FABP but not BNP was an independent prognostic predictor in patients with CHF.⁹ Niizeki et al also showed that H-FABP was the only independent predictor of CE in patients with CHF by multivariate analysis using 5 variables including BNP.²⁷ These results suggest that H-FABP is a new powerful prognostic predictor in CHF and we believe that based on the results of the present study the combination of TnT and H-FABP levels might be useful for risk stratification of CHF patients.

Study Limitations

H-FABP is a novel sensitive marker of myocardial damage but its level is significantly influenced by renal function.²⁸ In the present study, CHF patients with renal failure (creatinine >2.0 mg/dl) were excluded from the study (maximum creatinine level in CHF patients: 2.0 mg/dl). However, the creatinine levels in patients with high-H-FABP were significantly higher than those in patients with low-H-FABP. Eighteen of 25 patients (72%) with TnT+ and high-H-FABP and 21 of 27 (78%) patients with TnT- and high-H-FABP had normal renal function (creatinine ≤ 1.2 mg/dl). Furthermore, univariate analysis showed that creatinine >0.9 mg/dl was not a significant predictor of future CE. Multivariate analysis revealed that TnT+ and high-H-FABP were significant and independent predictors of future CE among 9 variables including creatinine. Renal dysfunction is an important prognostic predictors in CHF,^{29,30} but the results of this study suggest that the H-FABP level reflects membrane damage rather than the extent of renal dysfunction in CHF. Further investigation is required to determine the relationship between renal dysfunction and the H-FABP level.

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