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Coronary Microvascular Dysfunction and Diastolic Load Correlate With Cardiac Troponin T Release Measured by a Highly Sensitive Assay in Patients With Nonischemic Heart Failure

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Objectives This study investigated factors associated with cardiac troponin T (cTnT) release from failing myocardium.

Background Persistent and modest elevation of serum cTnT is frequently observed in heart failure (HF) patients free of coronary artery disease, although the mechanisms underlying this finding remain unclear.

Methods We evaluated serum cTnT levels in the aortic root (Ao) and coronary sinus (CS) using a highly sensitive assay in 90 nonischemic HF patients and 47 non-HF patients. Transcardiac cTnT and plasma B-type natriuretic peptide (BNP) release were described as the differences between CS and Ao cTnT levels [ΔcTnT (CS-Ao)] and BNP levels [ΔBNP (CS-Ao)], respectively. Coronary flow reserve (CFR) was measured in 68 HF patients using an intracoronary Doppler guidewire.

Results ΔcTnT (CS-Ao) levels were available in 76 HF patients and 28 non-HF patients (84% vs. 60%; $p = 0.001$), and higher in HF patients than non-HF patients ($p < 0.001$). Among HF patients, log[ΔcTnT (CS-Ao)] correlated with log[ΔBNP (CS-Ao)] ($r = 0.368$, $p = 0.001$), pulmonary capillary wedge pressure ($r = 0.253$, $p = 0.03$) and left ventricular end-diastolic pressure (LVEDP) ($r = 0.321$, $p = 0.005$). Multivariate regression analysis identified LVEDP as an independent parameter that correlated with ΔcTnT (CS-Ao). ΔcTnT (CS-Ao) levels were available in 58 HF patients who were evaluated for CFR. Coronary microvascular dysfunction, diagnosed by CFR <2.0, was observed in 18 HF patients. ΔcTnT (CS-Ao) was higher in patients with coronary microvascular dysfunction (4.8 [2.0 to 8.1] ng/l) than those without (2.0 [1.2 to 4.6] ng/l; $p = 0.04$).

Conclusions cTnT release from failing myocardium correlated with diastolic load and coronary microvascular dysfunction in nonischemic HF patients. (J Am Coll Cardiol 2013;62:632–40) © 2013 by the American College of Cardiology Foundation

Serum cardiac troponin I and T (cTnT) are sensitive and specific biomarkers for myocardial damage, and their measurement is recommended for accurate diagnosis of acute myocardial infarction (1). Elevated levels of serum cardiac troponin measured by conventional cardiac troponin assay correlate with adverse outcome in patients with both acute and chronic heart failure (HF) (2–4). However, in the majority of patients with HF, serum cardiac troponin levels are lower than the assay detection limits. Recently, a novel and highly sensitive cTnT (hs-TnT) assay has made it possible to detect low-grade circulating cTnT levels below the detection levels of conventional assays. This assay improves the diagnostic accuracy for myocardial infarction (5); prognostic discrimination, compared with conventional assays in patients with HF (6); and risk stratification for cardiovascular events and mortality in both patients with stable coronary artery disease (CAD) (7) and the general population (8–10).

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The levels of circulating cardiac troponin are modestly and persistently elevated in patients with stable HF, regardless of overt CAD (11). Various reasons have been proposed for the high serum troponin levels in patients with HF, including increased wall stress, myocyte damage from inflammatory cytokines or oxidative stress (11), altered calcium handling (12), and coronary microvascular dysfunction (CMVD) (13). At the molecular level, cardiac troponin is released from cardiomyocytes in response to myocardial necrosis, apoptosis, troponin degradation, increased membrane permeability, and stimulation of stretch-responsive integrins (11,14). Therefore, it is rational to speculate that persistent troponinemia may represent ongoing subclinical myocardial damage. However, this concept has not been demonstrated clinically, because serum cardiac troponin levels are affected by various clinical factors such as renal dysfunction (6,15), CAD (7,11), and skeletal muscle disease (16). In this study of patients with HF, we directly measured transcardiac cTnT release from nonischemic failing myocardium by hs-TnT assay to identify the factors associated with cTnT release, with a special focus on wall stress, inflammatory cytokines, oxidative stress, and CMVD.

Abbreviations and Acronyms

Ao = aortic root
CAD = coronary artery disease
CFR = coronary flow reserve
CI = confidence interval
CMVD = coronary microvascular dysfunction
CS = coronary sinus
cTnT = cardiac troponin T
FV = femoral vain
IL = interleukin
hs-CRP = high-sensitivity C-reactive protein
hs-TnT = highly-sensitive troponin T
LVEDP = left ventricular end-diastolic pressure
OR = odds ratio
TNF = tumor necrosis factor

Methods

Patient population. We evaluated 90 consecutive patients with nonischemic HF who underwent cardiac catheterization for evaluation of hemodynamics and coronary artery between January 2007 and May 2012 at Kumamoto University Hospital. Eligible patients were 20 years or older with HF consistent with the American College of Cardiology/American Heart Association Stage B or C classification (17). All patients were clinically stable and under optimal medical therapy for HF (18), including stable doses of an angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker, a beta-blocker, and an aldosterone blocker if not contraindicated. The control group comprised 47 non-HF patients who also underwent cardiac catheterization for suspected angina pectoris and were confirmed as normal by coronary angiography, which also showed no wall motion abnormality, cardiac hypertrophy, or valvular heart disease.

The following exclusion criteria were applied during patient selection: decompensated HF, ischemic HF, acute coronary syndrome, significant coronary artery stenosis (>50%), myocarditis, muscular dystrophy, polymyositis, implanted cardiac resynchronization device, and chronic inflammatory disease. Written informed consent was obtained from each patient before cardiac catheterization, and the study was approved by the ethics committee of our institution.

Cardiac catheterization and evaluation of coronary microvascular dysfunction. All examinations were conducted in the morning on fasting patients. In a total of 14 HF patients (16%), vasodilatory drugs, such as calcium channel blockers, nitrates, and nicorandil, were discontinued at least 72 h before examination to eliminate the vasodilatory effects of these drugs. A 5-F or 6-F arterial cannula was placed in the radial or femoral artery and a 6-F venous sheath was placed in the right femoral vein (FV). A coronary sinus (CS) catheter (Goodtec, Gifu, Japan) was advanced to the CS from the venous sheath, and its position was confirmed by fluoroscopy with contrast media. Blood samples were collected simultaneously from the aortic root (Ao), CS, and FV. Right heart catheterization was performed using a 6-F Swan-Ganz catheter (Fukuda Denshi, Tokyo, Japan) to measure hemodynamic parameters. Coronary angiography was then performed after administration of intracoronary isosorbide dinitrate to confirm the absence of significant epicardial coronary artery stenosis. After coronary angiography, the tip of a 0.014-inch Doppler-tipped guidewire (FloWire, Volcano, Rancho Cordova, California) was advanced to the proximal site of the left anterior descending coronary artery. Adenosine triphosphate (ATP) (150 μg/kg/min) was administrated intravenously until achievement of maximal hyperemia to calculate ATP-induced coronary flow reserve (CFR) (19). CFR ratios <2.0 were considered to indicate CMVD (13,19). The left ventricular end-diastolic pressure (LVEDP) was obtained, and left ventriculography was performed using a 5-F pigtail catheter.

Biomarker assays and measurement of cTnT release. The serum and plasma samples were kept frozen at −80°C until analysis. Serum cTnT levels were measured using the Elecsys 2010 Troponin T hs kit (Roche Diagnostics, Indianapolis, Indiana). The lower limit of detection is 5 ng/l with a reported 99th percentile value in apparently healthy individuals of 13.5 ng/l. At the 99th percentile value, the coefficient of variation is 9% by Elecsys 2010 analyzer (20). Plasma B-type natriuretic peptide (BNP) levels were measured using the MI02 Shionogi BNP kit (Shionogi, Osaka, Japan). cTnT and BNP release from the myocardium were calculated as the differences between CS and Ao levels of cTnT [ΔcTnT (CS − Ao)] and BNP [ΔBNP (CS-Ao)], respectively. Serum high-sensitivity C-reactive protein (hs-CRP), interleukin (IL)-6, and tumor necrosis factor (TNF)-α, representing inflammatory markers, were measured by the respective enzyme-linked immunosorbent assay (ELISA) (R&D systems, Minneapolis, Minnesota). Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) was measured by ELISA (8-OHdG check, Japan Institute for the Control of Aging, Shizuoka, Japan) as a marker of oxidative stress. The level of urinary 8-OHdG was corrected to urinary creatinine concentration.

Statistical analysis. Normally distributed data were presented as mean ± SD, whereas data of variables with skewed

distribution were expressed as medians with interquartile ranges. Differences between groups were examined by the Student *t* test or the Mann-Whitney *U* test for unpaired data. Categorical values were presented as numbers (percentage) and compared by the chi-square test or Fisher exact test as appropriate. Receiver-operating characteristic curves were generated to determine the cutoff value for the prediction of circulating cTnT level >13.5 ng/l. Variables with a skewed distribution were transformed logarithmically before Pearson's correlation and multiple regression analyses to fulfill the conditions required for the types of analysis. Linear relationships between ΔcTnT (CS-Ao) levels and key variables were first analyzed by univariate analysis

Table 1 Demographic and Clinical Characteristics of Participating Patients With Available Transcardiac cTnT Release

Variable	HF Group (n = 76)	Non-HF Group (n = 28)	p Value
Age, yrs	60 ± 14	63 ± 14	0.48
Male	49 (64%)	14 (50%)	0.18
Body mass index, kg/m^2	24.1 ± 3.9	24.3 ± 3.4	0.74
NYHA functional class, I/II/III/IV	23/45/8/0	NA	
Hypertension	42 (55%)	21 (75%)	0.07
Dyslipidemia	35 (46%)	21 (75%)	0.009
Diabetes mellitus	11 (15%)	6 (21%)	0.39
Atrial fibrillation	20 (26%)	2 (7%)	0.03
Prior HF hospitalization	15 (20%)	NA	
Hemodynamic status			
Cardiac index, l/min/m^2	2.39 ± 0.64	2.93 ± 0.87	0.001
PCWP, mm Hg	11.9 ± 4.9	9.3 ± 3.4	0.01
LVEDP, mm Hg	11.8 ± 6.8	8.2 ± 5.6	0.02
Medications			
Loop diuretics	28 (37%)	2 (7%)	0.003
Beta-blockers	56 (74%)	8 (29%)	<0.001
ACE-I or ARB	63 (83%)	12 (43%)	<0.001
Aldosterone antagonists	30 (40%)	0 (0%)	<0.001
Digitalis	7 (9%)	0 (0%)	0.19
Statins	22 (29%)	13 (46%)	0.09
Laboratory and imaging findings			
Ao-cTnT, ng/l	13.3 (8.9–21.0)	8.2 (6.3–10.4)	<0.001
FV-cTnT, ng/l	13.1 (9.3–19.4)	6.1 (5.1–10.4)	<0.001
Ao-BNP, pg/ml	114 (49–272)	15 (5–39)	<0.001
Hemoglobin, g/dl	14.0 ± 1.7	13.8 ± 1.9	0.51
Hemoglobin A_{1c}, %	6.0 ± 0.9	6.0 ± 0.6	0.81
Estimated GFR, ml/min/1.73 m^2	63.3 ± 19.4	69.2 ± 13.6	0.15
hs-CRP, mg/dl	0.08 (0.04–0.17)	0.03 (0.02–0.06)	<0.001
IL-6, pg/ml (n = 74)	1.37 (0.89–2.37)	NA	
TNF-alpha, pg/ml (n = 68)	1.93 (1.43–2.61)	NA	
8-OHdG, ng/mg creatinine (n = 47)	16.9 (11.9–26.8)	NA	
LVDd, mm	53.1 ± 8.7	45.3 ± 4.8	<0.001
LVEF, %	42.7 ± 12.1	64.7 ± 4.9	<0.001
E/e′	12.4 (9.3–15.4)	11.1 (8.6–13.5)	0.19
LV mass index, g/m^2*	160 ± 52	90 ± 19	<0.001
Etiology of heart failure			
Dilated cardiomyopathy	36 (47%)	NA	
Hypertrophic cardiomyopathy	13 (17%)	NA	
Hypertensive cardiomyopathy	12 (16%)	NA	
Cardiac amyloidosis	4 (5%)	NA	
Valvular heart diseases	3 (4%)	NA	
Others†	8 (11%)	NA	

Values are mean ± SD, n (%), or median (interquartile range). *LV mass index = {1.04 × [(IVST + LVDd + PWT)^3 − LVDd^3] × 0.8 + 0.6}/BSA. †Other etiologies included the following: tachycardia-induced cardiomyopathy (n = 1), diabetic cardiomyopathy (n = 1), mitochondrial cardiomyopathy (n = 2), LV noncompaction (n = 2), and unclassified cardiomyopathy (n = 2).

ACE-I = angiotensin-converting enzyme inhibitor; Ao-BNP = B-type natriuretic peptide level in the aortic root; Ao-cTnT = cardiac troponin T level in the aortic root; ARB = angiotensin II receptor blocker; BSA = body surface area; cTnT = cardiac troponin T; E/e′ = mitral early diastolic peak flow velocity to tissue Doppler early mitral annular diastolic velocity; FV = femoral vain; GFR = glomerular filtration rate; HF = heart failure; hs-CRP = high-sensitivity C-reactive protein; IL = interleukin; IVST = interventricular septal thickness; LV = left ventricular; LVDd = left ventricular end-diastolic dimension; LVEDP = left ventricular end-diastolic pressure; LVEF = left ventricular ejection fraction; NA = not available; NYHA = New York Heart Association; TNF = tumor necrosis factor; PCWP = pulmonary capillary wedge pressure; PWT = posterior wall thickness.

followed by stepwise multivariate analysis. A forward stepwise algorithm ($p < 0.10$ for entry, and $p < 0.05$ for stay) was used. Patients who were evaluated for CFR were divided into tertiles according to the ΔcTnT (CS-Ao) levels. Univariate and multivariate logistic regression analyses were performed to identify the independent parameters of highest tertile value. The Hosmer-Lemeshow statistic was applied to assess model calibration. A 2-tailed value of $p < 0.05$ was considered statistically significant. All statistical analyses were performed with SPSS version 19 (SPSS, Chicago, Illinois).

Results

cTnT measurement and comparison of HF and control groups. The serum levels of cTnT in Ao (Ao-cTnT) were detectable in 80 HF patients and 28 non-HF patients (89% vs. 60%, respectively; $p < 0.001$). Plasma BNP levels in Ao (Ao-BNP) was significantly higher in HF patients with detectable levels of serum cTnT compared with those with undetectable levels (114 [51 to 272] pg/ml vs. 36 [11 to 92] pg/ml; $p = 0.007$). Age (63 ± 13 years vs. 57 ± 9 years; $p = 0.09$) and LVEDP (8.2 ± 5.6 mm Hg vs. 5.0 ± 3.8 mm Hg; $p = 0.054$) tended to be higher in non-HF patients with detectable levels of serum cTnT compared with those with undetectable levels. The levels of serum cTnT in FV (FV-cTnT), representing the circulating cTnT levels, correlated strongly with Ao-cTnT ($r = 0.954$, $p < 0.001$). The ratio of transcardiac cTnT release to FV-cTnT level was 18.8 ± 14.8% in the study patients with detectable Ao-cTnT. Receiver-operating characteristic analysis selected 2.2 ng/l as the best cutoff value of ΔcTnT (CS-Ao) level for the prediction of circulating cTnT level >13.5 ng/l, representing the 99th percentile cutoff value of this assay, with sensitivity and specificity of 61% and 71%, respectively, and area under the curve of 0.66 (95% confidence interval [CI]: 0.55 to 0.77; $p = 0.006$). Similarly, 75.4 pg/ml was the best cutoff value of Ao-BNP level for the prediction of circulating cTnT level >13.5 ng/l with a sensitivity and specificity of 61% and 66%, respectively, and area under the curve of 0.70 (95% CI: 0.60 to 0.80; $p = 0.001$).

The serum cTnT levels in CS (CS-cTnT) were higher than those of Ao-cTnT in 76 HF patients (95%) and all non-HF patients with detectable Ao-cTnT. These results suggested the availability of transcardiac cTnT release in these patients. Table 1 details the baseline characteristics of the study patients with available levels of ΔcTnT (CS-Ao). Among the 76 HF patients with available ΔcTnT (CS-Ao), IL-6 and TNF-α were measurable in 74 patients. IL-6 was detected in all patients (limit of detection: 0.156 pg/ml), whereas TNF-α was detected in 68 patients (limit of detection: 0.5 pg/ml). Urinary 8-OHdG was measurable in 47 patients.

ΔcTnT (CS-Ao) (2.5 [1.2 to 4.9] ng/l) and ΔBNP (CS-Ao) (235 [90 to 382] pg/ml) levels of the HF patients were significantly higher than those of the non-HF group (1.2 [0.5 to 1.4] ng/l; $p < 0.001$, and 28 [10 to 58] pg/ml; $p < 0.001$, respectively) (Fig. 1).

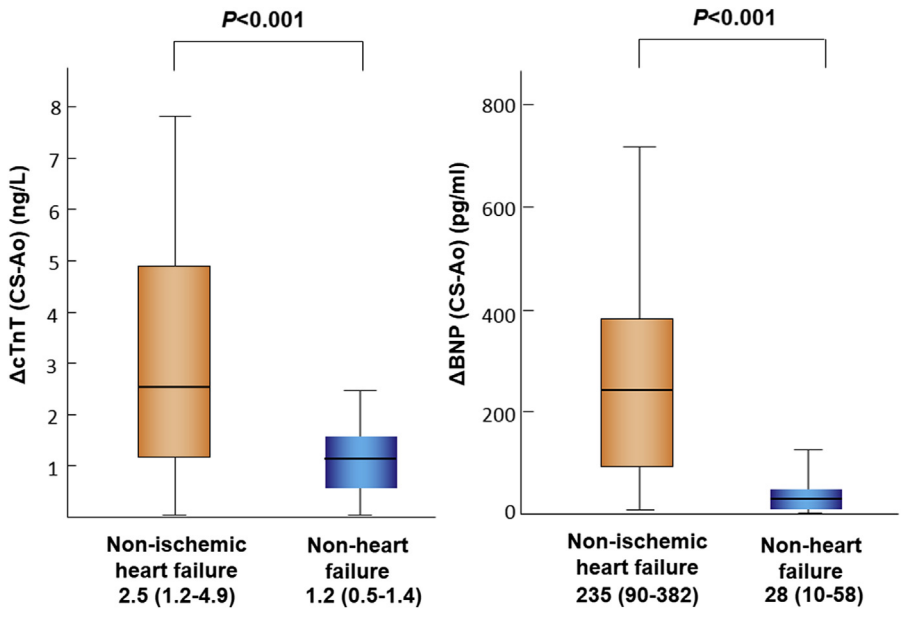

Figure 1 Comparison of cTnT and BNP Release Between Nonischemic HF Patients and Non-HF Group

ΔcTnT (CS-Ao) and ΔBNP (CS-Ao) levels were significantly higher in nonischemic HF patients than in non-HF group. Data are median and interquartile ranges. ΔBNP (CS-Ao) = (BNP level in coronary sinus) − (BNP level in aortic root); ΔcTnT (CS-Ao) = (cTnT level in coronary sinus) − (cTnT level in aortic root); BNP = B-type natriuretic peptide; cTnT = cardiac troponin T.

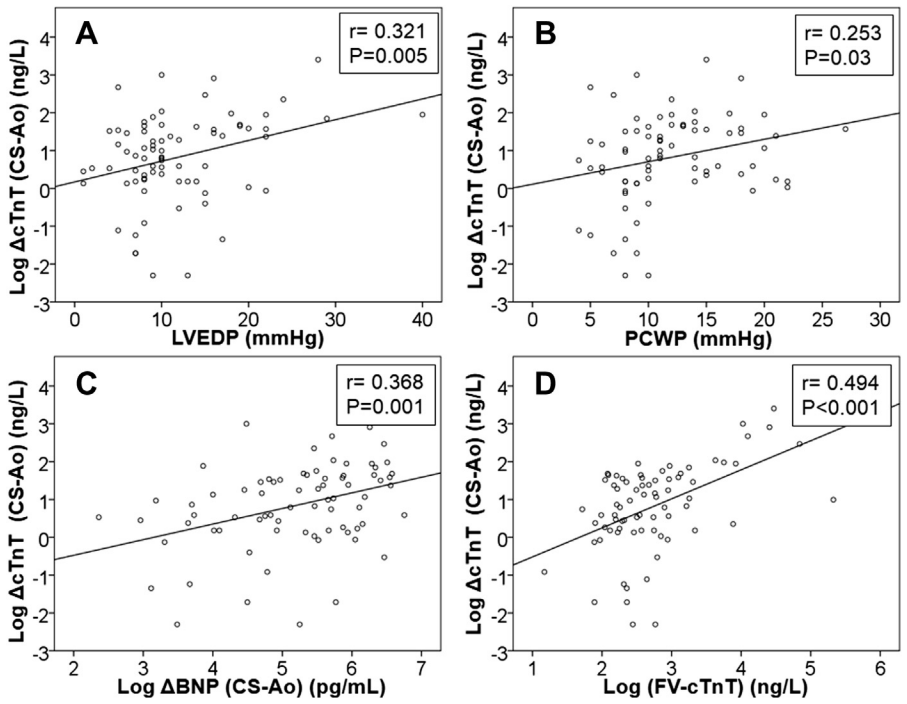

Figure 2 Correlation of Log [ΔcTnT (CS-Ao)] With LVEDP, PCWP, Log [ΔBNP (CS-Ao)], and Log (FV-cTnT) in Patients With Nonischemic HF

LVEDP **(A)**, PCWP **(B)**, log [ΔBNP (CS-Ao)] **(C)**, and log (FV-cTnT) **(D)** levels correlated positively with log [ΔcTnT (CS-Ao)]. FV-cTnT = cTnT level in femoral vein; LVEDP = left ventricular end-diastolic pressure; PCWP = pulmonary capillary wedge pressure; other abbreviations as in Figure 1.

Evaluation of factors associated with cTnT release. Among the 76 HF patients with available ΔcTnT (CS-Ao), log [ΔcTnT (CS-Ao)] levels correlated positively with pulmonary capillary wedge pressure (r = 0.253, p = 0.03), LVEDP (r = 0.321; p = 0.005), log (FV-cTnT) (r = 0.494; p < 0.001), log (Ao-BNP) (r = 0.266, p = 0.02), and log [ΔBNP (CS-Ao)] (r = 0.368, p = 0.001) (Fig. 2). However, cTnT release did not correlate with markers of inflammation (hs-CRP, IL-6, and TNF-α) or oxidative stress (8-OHdG) (Table 2). To examine the factors associated with cTnT release, we performed stepwise multiple regression analysis. The analysis identified LVEDP (β = 0.321, p = 0.006) (Table 2) as the only independent and significant parameter that correlated with ΔcTnT (CS-Ao).

Table 2 Results of Univariate and Multivariate Linear Regression Analyses for Log [ΔcTnT (CS-Ao)]

	Univariate Analysis		Multivariate Analysis	
Variable	β	p Value	β	p Value
Age	−0.192	0.10	Not selected	
Body mass index	0.068	0.56	Not selected	
Cardiac index	−0.034	0.77	Not selected	
PCWP	0.253	0.03	—	
LVEDP	0.321	0.005	0.321	0.006
LVEF	0.126	0.28	Not selected	
Log (E/e′)	0.151	0.20	Not selected	
LV mass index	0.076	0.51	Not selected	
Hemoglobin	0.184	0.11	Not selected	
Hemoglobin A_{1c}	0.003	0.98	Not selected	
Estimated GFR	0.039	0.74	Not selected	
Log (FV-cTnT)	0.494	<0.001	—	
Log (Ao-BNP)	0.266	0.02	—	
Log [ΔBNP (CS-Ao)]	0.368	0.001	—	
Log (hs-CRP)	0.170	0.15	Not selected	
Log (IL-6)	0.009	0.94	—	
Log (TNF-alpha)	0.036	0.77	—	
Log (8-OHdG)	0.004	0.98	—	

"Not selected" indicates not selected by stepwise algorithm.
Abbreviations as in Table 1.

High cTnT release in HF patients with CMVD. CFR was evaluated in 68 HF patients (76%). Among them, ΔcTnT (CS-Ao) was available in 58 HF patients (85%). Log [ΔcTnT (CS-Ao)] levels did not correlate with actual CFR values (r = −0.054, p = 0.69). CMVD, reflected by CFR <2.0, was observed in 18 HF patients with available ΔcTnT (CS-Ao) (31%). Ao-cTnT, Ao-BNP, ΔBNP (CS-Ao) levels, and left ventricular mass index were significantly higher and hemoglobin was lower in patients with CMVD than in those without (Table 3), as were ΔcTnT (CS-Ao) levels (4.8 [2.0 to 8.1] ng/l vs. 2.0 [1.2 to 4.6] ng/l; p = 0.04) (Fig. 3). The following variables were entered into stepwise multiple regression analysis to identify the factors associated with cTnT release: age, sex, the presence of CMVD, log(hs-CRP), hemoglobin, left ventricular ejection fraction, and left ventricular mass index. The analysis

Table 3 Clinical Characteristics of Patients With and Without CMVD

Variable	CFR <2.0 (n = 18)	CFR ≥2.0 (n = 40)	p Value
Age, yrs	60 ± 15	61 ± 15	0.93
Male	12 (67%)	31 (78%)	0.52
Body mass index, kg/m^2	24.0 ± 4.0	24.2 ± 3.7	0.88
NYHA functional class	2.1 ± 0.4	1.8 ± 0.7	0.045
Hypertension	11 (61%)	23 (58%)	0.80
Dyslipidemia	7 (39%)	19 (48%)	0.54
Diabetes mellitus	4 (22%)	6 (15%)	0.48
Cardiac index, l/min/m^2	2.42 ± 0.69	2.33 ± 0.67	0.67
PCWP, mm Hg	11.7 ± 3.7	12.7 ± 5.1	0.49
LVEDP, mm Hg	12.9 ± 7.1	11.4 ± 5.9	0.39
CFR	1.6 ± 0.3	3.3 ± 0.9	<0.001
Baseline average peak flow velocity, cm/s	17.0 (14.0–36.0)	15.5 (12.0–20.0)	0.06
Hyperemic average peak flow velocity, cm/s	30.0 (22.5–60.5)	48.0 (37.5–62.5)	0.02
Ao-cTnT, ng/l	24.8 (14.3–50.0)	12.2 (8.3–17.6)	0.003
Ao-BNP, pg/ml	354 (149–519)	75 (33–196)	<0.001
ΔBNP (CS-Ao), pg/ml	451 (273–569)	179 (96–304)	0.003
Hemoglobin, g/ml	13.4 ± 1.9	14.6 ± 1.5	0.008
Hemoglobin A_{1c}, %	6.2 ± 1.3	6.0 ± 0.9	0.53
Estimated GFR, ml/min/1.73 m^2	59.2 ± 24.9	65.1 ± 17.9	0.31
hs-CRP, mg/dl	0.10 (0.06–0.32)	0.08 (0.03–0.16)	0.09
IL-6, pg/ml	1.53 (0.99–3.53)	1.37 (0.78–2.32)	0.40
TNF-alpha, pg/ml	2.10 (1.32–2.34)	1.94 (1.42–2.61)	0.76
8-OHdG, ng/mg creatinine	28.4 (10.6–39.6)	16.1 (11.0–19.7)	0.17
LVDd, mm	54.2 ± 10.8	53.7 ± 8.2	0.85
LVEF, %	42.1 ± 10.7	41.1 ± 11.5	0.76
E/e′	14.9 (10.1–17.3)	12.6 (9.4–14.9)	0.27
LV mass index, g/m^2	186 ± 57	152 ± 51	0.03
Etiology of heart failure			
Dilated cardiomyopathy	6 (33%)	20 (50%)	
Hypertrophic cardiomyopathy	3 (17%)	6 (15%)	
Hypertensive cardiomyopathy	2 (11%)	9 (23%)	
Cardiac amyloidosis	4 (22%)	0	
Valvular heart disease	1 (6%)	1 (3%)	
Others	2 (11%)	4 (10%)	

Values are mean ± SD, n (%), or median (interquartile range).
CMVD = coronary microvascular dysfunction; CRF = coronary flow reserve; other abbreviations as in Table 1.

identified the presence of CMVD (β = 0.322, p = 0.02) as an independent and significant parameter associated with ΔcTnT (CS-Ao). Multivariate logistic regression analysis was performed to identify the independent parameters of highest tertile value of ΔcTnT (CS-Ao) (cutoff value >4.2 ng/l) among the 68 HF patients who were evaluated for CFR. The analysis identified LVEDP (odds ratio [OR]: 1.10; 95% CI: 1.00 to 1.21; p = 0.04), and the presence of CMVD (OR: 6.60; 95% CI: 1.75 to 24.97; p = 0.005) as 2 independent and significant correlates with highest tertile value of ΔcTnT (CS-Ao) (Table 4).

Discussion

To the best of our knowledge, this is the first report that measured transcardiac cTnT release from nonischemic failing myocardium by high-sensitivity assay. The major findings of the present study were: 1) high ΔcTnT (CS-Ao) levels in nonischemic HF patients compared with non-HF patients; 2) ΔcTnT (CS-Ao) levels were significantly higher in patients with CMVD than those without; and 3) LVEDP and the presence of CMVD were significant and independent determinants of ΔcTnT (CS-Ao) levels. Together, these findings indicate that increased diastolic load and CMVD seem to be the causes of persistent and modest cTnT release from the failing myocardium.

Serum cardiac troponin level is influenced by various clinical factors such as renal clearance (6,15), presence of CAD (7,11), and skeletal muscle disease (16). To exclude the involvement of these factors, we evaluated cTnT release directly by measuring the differences in cTnT levels between CS and Ao in nonischemic HF patients. Tsutamoto et al. (15) measured serum cTnT level in the Ao and CS by conventional assay in HF patients, but detected Ao-cTnT in only 37% of patients (the detection limit of this assay was >10 ng/l). Moreover, CS-cTnT levels were lower than

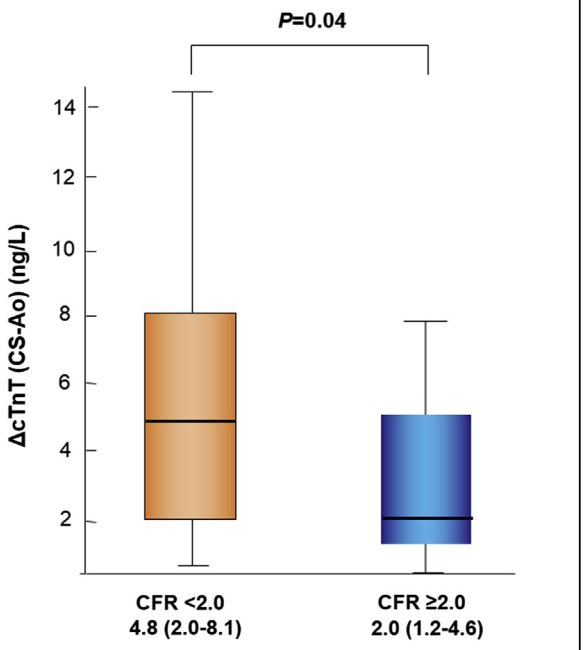

Figure 3 **Comparison of cTnT Release in the Presence and Absence of Coronary Microvascular Dysfunction**

CFR ratio <2.0 indicates existing coronary microvascular dysfunction. ΔcTnT (CS-Ao) levels were significantly higher in patients with coronary microvascular dysfunction than in those without. Data are medians and interquartile ranges. CFR = coronary flow reserve; other abbreviations as in Figure 1.

Ao-cTnT levels in 39% of patients tested. These data imply that persistent and low-grade transcardiac cTnT release in stable HF patients cannot be evaluated precisely by the conventional assay. By comparison, we were able to detect Ao-cTnT and positive ΔcTnT (CS-Ao) at high frequency (89% and 95%, respectively) in our study group of HF patients using the highly sensitive assay. In addition to measuring troponin release, we recorded hemodynamics simultaneously by right heart catheterization and evaluated circulating cTnT levels and coronary microvascular function using an intracoronary Doppler guidewire, enabling us to evaluate precisely the factors associated with cTnT release from the failing myocardium.

Elevated cardiac troponin levels correlate with elevated BNP levels and impaired hemodynamics in patients with HF (11,21). Kusumoto et al. (22) measured circulating cTnT levels by highly sensitive assay in HF patients and described a positive correlation between circulating BNP and cTnT levels. However, they did not eliminate the effect of confounding factors, such as renal function and myocardial ischemia. By comparison, we examined transcardiac cTnT release to identify the mechanism of its release after excluding these confounding factors in nonischemic HF patients. Therefore, our methodology and purpose are novel and definitely different from the aforementioned study. In the present study, univariate analysis confirmed that cTnT release correlated positively with BNP release. These results suggest that cTnT release is associated with wall stress in the failing myocardium because BNP is released from the heart in response to volume expansion and pressure overload associated with wall stress (23). Moreover, previous studies reported that circulating cTnT levels correlated positively with N-terminal pro-BNP levels even in non-HF patients (8). In our study, BNP release also correlated positively with cTnT release in non-HF patients similar to HF patients ($r = 0.546$, $p = 0.01$). Thus, we speculate that transcardiac cTnT release in non-HF patients could be potentially a signal of subtle increase in wall stress though inadequate to provoke HF symptoms.

How does wall stress in the absence of myocardial necrosis change cardiac troponin release? Although the exact mechanism is unknown at this stage, several mechanisms have been postulated. Hessel et al. (14) reported that intact cardiac troponin I was released from viable cardiomyocytes on stimulation of the stretch-responsive integrins without an increase in lactate production, inferring that release can occur without ischemia or necrosis. Furthermore, Clarke et al. (24) reported that mechanical stimuli might produce transient disruption of the myocardial plasma membrane, suggesting increased membrane permeability as a potential mechanism. These reports and our present findings indicate that stretch and mechanical stress of cardiac myocytes might lead to leakage of the intact troponin from the cytosolic pool with a transient loss of cell membrane integrity and increased membrane permeability. However, it remains unclear whether the threshold level and amount of released cardiac troponin are different between normal heart and failing heart under these circumstances, and further studies are needed to clarify this point.

Activation of inflammatory cytokines and oxidative stress are presumed to increase serum cTnT level in HF (11); however, our study showed these factors to be unrelated to cTnT release. This could be related to the patient selection criteria applied in the present study; the majority of our patients had only mild symptoms (89% were New York Heart Association functional class I or II) and were under optimal medication. Because inflammatory cytokines and oxidative stress increase with the progression of HF (25,26), the association between cTnT release and these factors might be applicable only to patients with advanced HF. On the other hand, statins and carvedilol are reported to reduce inflammation and oxidative stress (27,28). However, in this study, there was no difference in cTnT release and inflammatory cytokine levels between subjects treated with or without statins and beta-blockers (data not shown). Nevertheless; we cannot rule out possible interference of these medications with our results.

CMVD is an established feature of nonischemic cardiomyopathies, which can also exacerbate HF and worsen prognosis (29,30). Several mechanisms have been proposed to explain the development of CMVD in HF, such as increased extramural compression due to elevated LVEDP, endothelial dysfunction, vascular remodeling, and decreased

Table 4 Results of Univariate and Multivariate Logistic Regression Analyses for Parameters That Correlated with Highest Tertile Value of ΔcTnT (CS-Ao)

Variables	Univariate Analysis		Multivariate Analysis	
	p Value	OR (95% CI)	p Value	OR (95% CI)
Age, per yr	0.59	0.99 (0.96–1.02)	Not selected	
Male, yes	0.74	0.83 (0.27–2.51)	Not selected	
Body mass index, per kg/m^2	0.88	1.01 (0.89–1.14)	Not selected	
NYHA functional class III, yes	0.47	2.11 (0.41–6.99)	Not selected	
Hypertension, yes	0.35	1.68 (0.58–4.63)	Not selected	
Dyslipidemia, yes	0.93	1.05 (0.38–2.87)	Not selected	
Diabetes mellitus, yes	0.28	0.46 (0.12–1.86)	Not selected	
Cardiac index, per l/min/m^2	0.89	0.95 (0.43–2.07)	Not selected	
PCWP, per mm Hg	0.14	1.09 (0.97–1.22)	—	
LVEDP, per mm Hg	0.01	1.11 (1.02–1.21)	0.040	1.10 (1.00–1.21)
Presence of CMVD, yes	0.006	4.98 (1.58–15.69)	0.005	6.60 (1.75–24.97)
ΔBNP (CS-Ao), per pg/ml	0.007	1.00 (1.00–1.01)	—	
Hemoglobin, per g/dl	0.79	1.04 (0.78–1.39)	Not selected	
Estimated GFR, per ml/min/1.73 m^2	0.92	1.00 (0.98–1.03)	Not selected	
Hemoglobin A_{1c}, per %	0.76	0.93 (0.58–1.49)	Not selected	
hs-CRP, per mg/dl	0.87	0.83 (0.10–7.23)	Not selected	
LVEF, per %	0.37	1.02 (0.98–1.07)	Not selected	
E/e′, per 1.00	0.77	1.02 (0.92–1.12)	Not selected	
LV mass index, per g/m^2	0.42	1.00 (0.99–1.01)	Not selected	

"Not selected" indicates not selected by stepwise algorithm.
CI = confidence interval; OR = odds ratio; other abbreviations as in Tables 1 and 3.

myocardial capillary density (13,31). Indeed, CMVD impairs myocardial perfusion and induces regional metabolic changes as in recurrent or persistent myocardial ischemia (32,33). In the present study, we demonstrated that elevated LVEDP and CMVD were associated with cTnT release. Thus, it is possible that the elevated LVEDP is linked to CMVD through the resultant compression of myocardial capillaries, with subsequent decrease in their lumina, leading to increased myocardial contractility, which in turn can cause functional myocardial ischemia and cTnT release.

Are our findings important clinically? Because increased LVEDP and CMVD are modifiable targets that can reduce cTnT release, interventions aimed at improving these factors should prevent exacerbation of HF and worsening prognosis. In the present study, we did not evaluate cTnT release in decompensated or acute HF; however, we assume that left ventricular diastolic load and CMVD potentially enhance cTnT release in decompensated situation. High circulating cTnT levels result in impaired hemodynamics, and serial measurements may be useful for the assessment of left ventricular pressure overload, functional myocardial ischemia, and effects of therapeutic intervention.

Study limitations. An important point to consider with the study findings is that HF etiology could be heterogeneous and the mechanisms of cTnT release might differ among cardiomyopathies. In addition, this study investigated only a relatively small number of patients in a single center and was limited to patients with nonischemic and well-compensated HF. Further large studies including decompensated HF patients are warranted to confirm our observations. Several studies reported that vasodilators affect coronary microcirculation (34,35). Thus, we discontinued these drugs to eliminate any exogenous effect on the coronary microcirculation. On the other hand, it was reported that discontinuation of these drugs could affect the hemodynamics and alter endothelial function (36). Although the number of our patients who required discontinuation of their medications was relatively small (16%) and such patients had enough drug holidays (>72 h), we cannot exclude a possible rebound phenomenon and its effect on our results. Finally, we evaluated CMVD by CFR of only the left anterior descending coronary artery area; however, cardiomyopathy is characterized by global myocardial dysfunction, and we assumed that CMVD would be homogenously distributed throughout the myocardium.

Conclusions

The present study demonstrated an increased release of cTnT from the myocardium of nonischemic HF patients compared with non-HF patients, and such an increase correlated with increased left ventricular diastolic load and CMVD. These factors could contribute to persistent and modest cTnT elevation in nonischemic HF patients, exacerbate HF, and worsen prognosis. Thus, therapeutic or preventive interventions aimed at improving these factors to reduce cTnT release in patients with HF should be applied to escape the vicious cycle of worsening HF syndrome.

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