

Association between matrix metalloproteinase-9 and worsening heart failure events in patients with chronic heart failure

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Abstract

Aims Matrix metalloproteinase (MMP) is up-regulated during heart failure (HF) and influences ventricular remodeling. We hypothesized that disparity between MMP-9 and tissue inhibitors of MMP-1 (TIMP-1) results in clinical manifestations and is related to prognostic risk in patients with chronic HF.

Methods and results Plasma levels of MMP-9, TIMP-1, and brain natriuretic peptide (BNP) were measured in 173 patients with chronic HF. Combined endpoints of worsening HF events were assessed during follow-up (median 109 months). MMP-9 and TIMP-1 levels and the MMP-9/TIMP-1 ratio increased with increasing severity of the New York Heart Association class (P for trend = 0.003, 0.011, and 0.005, respectively). Patients with HF events ($n = 35$) had significantly higher MMP-9 than those without HF events ($P = 0.004$). Kaplan–Meier analysis demonstrated a higher probability of HF events with high MMP-9 values (>23.2 ng/mL; $P = 0.005$). A multivariate Cox proportional hazard model showed that high MMP-9 values were an independent predictor of HF events (hazard ratio, 3.73; 95% confidence interval (CI), 1.03–13.46; $P = 0.043$). In patients with lower BNP levels (≤ 210 pg/mL), the adjusted hazard ratio for HF events was 3.63 (95% CI, 1.20–11.02; $P = 0.023$) among patients with high MMP-9 values compared with patients with low BNP and low MMP-9 values.

Conclusions MMP-9 and TIMP-1 levels correlate with the severity of chronic HF. MMP-9 is a strong predictor of HF events, suggesting that a disparity between MMP-9 and TIMP-1 levels and increased MMP-9 levels may help predict HF events.

Keywords Matrix metalloproteinase-9; Tissue inhibitors of matrix metalloproteinase-1; Chronic heart failure; Brain natriuretic peptide

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Introduction

Cardiac remodeling is accompanied by an extensive reorganization of the cardiac extracellular matrix. Metalloproteinases (MMPs) are zinc-dependent endopeptidases that catalyze degradation of extracellular matrix proteins, thereby controlling such processes as development and tissue remodeling.^{1–3}

MMPs play an important role in many pathophysiological processes like angiogenesis and in diseases with a degradation component such as heart failure (HF), myocardial infarction and atherosclerotic plaque. The activity of MMPs is

controlled by regulation of expression and proteolytic activation of pro-enzymes and by the Tissue Inhibitors of Metalloproteinases (TIMPs). TIMP-1 binds to the inactive pro-MMP-9, forming a complex in which TIMP-1 retains its ability to inhibit the activity of another active MMP. Imbalance of MMP and TIMP activity may correlate with pathological conditions in patients with HF. MMPs and TIMPs are matrix-degrading enzymes that are up-regulated in the failing heart and influence left ventricular remodeling.^{2–4} Recently, several biomarkers have been identified that can predict an adverse prognosis in patients with HF. Of these, elevated plasma concentrations of brain natriuretic peptide

(BNP) show notable associations with development of left ventricular dysfunction and HF.⁵ Although elevated BNP concentrations have diagnostic and prognostic importance,^{6–8} whether intense therapy for HF guided by BNP values is superior to therapy guided by clinical symptoms is unclear.^{9–11} BNP play important roles in maintaining cardiorenal homeostasis under physiological and pathological conditions. BNP is synthesized by cardiomyocytes and fibroblasts, and their production is stimulated in pathologic conditions such as myocardial infarction and HF. Additionally, BNP inhibits collagen synthesis and increases MMP expression.¹² Proinflammatory cytokines such as IL-6 and TNF- α also play an important role in cardiac remodeling.^{13,14} Induction or stimulation of MMP expression is mediated by a variety of inflammatory cytokines, including IL-6 and TNF- α .¹⁵ During the progression of left ventricular dysfunction, increased sympathetic stimulation and levels of norepinephrine were observed. Following chronic neurohormonal system activation, increased elaboration and release of MMPs into the extracellular matrix of the left ventricle occurs.¹⁶ Therefore, the sympathetic nerve system may modulate MMP productions in patients with HF. Thus, we evaluated the significance of circulating MMP and TIMP levels and comprehensive neurohormonal profiles in patients with chronic HF.

We tested the hypothesis that an imbalance between MMP-9 and TIMP-1 levels is related to prognostic risk in patients with chronic HF. We examined whether circulating MMP molecules are related to the prognostic risk of chronic HF and determined the prognostic risk of HF events associated with circulating levels of MMP-9, TIMP-1, and other biomarkers.

Methods

Study subjects and design

Patients were recruited from the Department of Cardiovascular Medicine of the University of Fukui Hospital. Patients with chronic HF ($n = 202$) were enrolled in this study. Symptoms and signs of HF were defined according to the Framingham criteria.¹⁷ Twenty-nine patients were excluded due to exclusion criteria as stated as follows and/or because their blood samples were not appropriate for measuring. We also excluded patients with a history of neoplastic, hepatic, infectious, or autonomic disease; peripheral atherosclerotic disease; or any surgical procedure in the preceding 6 months. No patients had inflammatory signs at the time of evaluation. Thus, 173 inpatients who were admitted between June 1, 1992 and August 31, 2002 were enrolled. The investigation conformed to the principles outlined in the Declaration of Helsinki. Ethics committee approval and informed consent from all patients were obtained.

To obtain follow-up information and screen for the occurrence of HF events, all patients were reevaluated after discharge from the hospital by reviewing their medical records including clinical visits, conducting telephone interviews, and obtaining clinical information from the in-charge cardiologists; the last follow-up was on December 31, 2009. We observed the HF events that occurred during the follow-up period (mean \pm SD, 88 ± 49 months) and looked for a correlation between disease severity and events, and between TIMP-1, BNP, noradrenaline (NA), interleukin [IL]-6 and tumor necrosis factor [TNF]- α and MMP levels during follow-up. HF events were defined as cardiac death (including sudden cardiac death and death from HF), lethal arrhythmia (ventricular tachycardia/ventricular fibrillation), and admission due to worsening HF. Patients were classified into two groups: patients with HF events ($n = 35$) and patients without HF events ($n = 138$). HF with reduced ejection fraction (HFREF) was defined as a left ventricular ejection fraction $\leq 45\%$, whereas HF with preserved ejection fraction (HFPEF) was defined as a left ventricular ejection fraction $> 45\%$.

Blood collections and plasma separation

All patients were treated with standard regimens for HF, and all drugs continued to be administered before blood collection. Overnight fasting blood samples were obtained from the forearm or femoral vein and kept on ice. Plasma and serum samples were separated by centrifugation within 30 min, frozen, and stored at -80°C until use. BNP and NA were assayed rapidly after blood collection. MMP family members and cytokines were assayed within 1 year after blood collection. Samples used for analysis had not been previously thawed. On the day of use, the samples were thawed at room temperature and analysed immediately.

Measurements of plasma MMP-9 and serum TIMP-1 levels

Sandwich enzyme immunoassays using commercially available kits with monoclonal antibodies against each molecule were performed to measure the concentrations of plasma MMP-9 and serum TIMP-1 according to the manufacturer's instructions (Fuji Chemical Industries Ltd., Takaoka, Japan).¹⁸

Measurements of plasma brain natriuretic peptide and noradrenaline

Plasma levels of BNP were measured using a commercially available radioimmunoassay kit (Shionogi, Osaka, Japan), and plasma levels of NA were measured with high-

performance liquid chromatography.⁶ Plasma NA concentration provides prognosis utility in congestive HF patients.¹⁹

Measurement of plasma levels of cytokines

Sandwich enzyme immunoassays using commercially available kits with monoclonal antibodies against each molecule were performed to measure concentrations of plasma cytokines (IL-6 and TNF- α) according to the manufacturer's instructions (Quantikine HS, R&D Systems, Minneapolis, MN, USA). The same trained researcher, who was blinded to the patient's clinical status, performed all tests throughout the study. Detailed methods are as previously reported.²⁰ TNF- α and IL-6 are elevated in patients with HF, and these cytokines are correlated with both the severity and development of HF. Thus, these cytokines may have both pathophysiological importance and utility as clinically predictive biomarkers.

Statistical analysis

Continuous values are expressed as the mean \pm SD or median [interquartile range]. The chi-square test was used to compare categorical data. Comparisons between two groups of continuous measurements were performed with Mann-Whitney U tests. Correlations between two variables were assessed with the Spearman rank test. Comparisons of MMP-9 and TIMP-1 levels, and the MMP-9/TIMP-1 ratio across New York Heart Association (NYHA) functional classes were performed with the Jonckheere-Terpstra trend test and Kruskal-Wallis analysis of variance. Receiver operating characteristic (ROC) curve analysis was used to determine the ability of the variables to distinguish between patients with and without HF events. Optimal cut-off values for each variable were calculated to provide the greatest combined sensitivity and specificity. A Cox proportional hazards regression model was used to explore the effect of other variables on event-free survival. Variables included in the model were age, sex, calcium channel blockers, BNP, NA, TNF- α , MMP-9, TIMP-1, IL-6, and ejection fraction. Hazard ratios (HR) and 95% confidence intervals (CI) were determined. With regard to sample size estimation, we planned a study with an accrual interval of 120 months, and additional follow-up after the accrual interval of 84 months. The median survival time on the low MMP group was estimated as 84 months. If the true hazard ratio (relative risk) of low MMP subjects relative to high MMP subjects is 2, we will need to study 61 experimental subjects and 61 control subjects to be able to reject the null hypothesis that the experimental and control survival curves are equal with probability (power) 80%. The Type I error probability associated with this test of this null hypothesis is 0.05.

We generated Kaplan-Meier curves to describe the association between MMP-9 values and time to incident HF events. Log rank tests were used to compare event-free survival between different groups. We assessed the model performance of the modified model, which was made by incorporating MMP-9 information into the model. Variables included in the model were age, sex, calcium channel blockers and BNP. Additive information of MMP-9 was evaluated by category-free net reclassification improvement (NRI) and integrated discrimination improvement (IDI). Statistical significance was set at $P < 0.05$ (two-tailed). Data were analysed with R software V.3.0.1 (<http://www.r-project.org>) and SPSS version 20 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

Baseline demographics, medications, and etiology of HF did not differ between the group of patients with and without HF events. Compared with the non-HF events group, the HF events group included patients with higher MMP-9, TIMP-1, IL-6, and TNF- α levels. Patients with HF events also had a lower ejection fraction and higher prescription rate of calcium channel blockers. During the follow-up period, 35 patients experienced HF events. Patients who had HF events had higher MMP-9 levels than patients without HF events (28.0 [17.4–50.5] ng/mL vs. 20.0 [14.3–33.6] ng/mL, $P = 0.004$) (*Table 1*). When patients were classified into two groups: patients with symptomatic HF (classified as NYHA class II, III, and IV) ($n = 104$) and patients without symptomatic HF (classified as NYHA class I) ($n = 69$), we observed higher MMP-9 levels in patients with symptomatic HF than in patients without symptomatic HF (23.1 [15.7–45.8] ng/mL vs. 20.1 [14.0–26.5] ng/mL, $P = 0.04$). No significant differences were found between symptomatic HF and asymptomatic HF in levels of TIMP-1 (134.5 [67.5–174.0] ng/mL vs. 116.0 [45.0–159.7] ng/mL, $P = 0.08$).

Sixty-four patients were classified as HFREF, and 109 patients were classified as HFPEF. We observed higher plasma levels of BNP in HFREF than in HFPEF (278.0 [9.6–769.7] pg/mL vs. 81.6 [5.0–217.7] pg/mL, $P < 0.01$) and a higher MMP-9/TIMP-1 ratio in HFREF than in HFPEF (0.242 [0.073–0.665] vs. 0.179 [0.041–0.421], $P = 0.03$). No significant differences were found between HFPEF and HFREF in levels of MMP-9 (21.7 [4.5–41.6] ng/mL vs. 25.7 [1.0–68.2] ng/mL, $P = 0.09$) or TIMP-1 (138.0 [66.6–209.4] ng/mL vs. 160.0 [89.1–230.9] ng/mL, $P = 0.06$) (see Supporting Information *Figure S1*). There were no significant interactions between MMP-9 and TIMP-1 levels and following drugs administration; beta-blocker, ARB/ACE-I, statin, calcium channel blocker, nitrate, digitalis.

Table 1 Patient characteristics

	Chronic HF (n = 173)	HF events (+) (n = 35)	HF events (-) (n = 138)	P value
Age, years	64.4 ± 12.4	65.0 ± 16.2	64.3 ± 11.4	0.27
Sex (M/F), n (%)	114 (65.8)/59 (34.1)	22 (62.9)/13 (37.1)	92 (66.7)/46 (33.3)	0.69
Hypertension, n (%)	93 (53.7)	15 (42.9)	78 (56.5)	0.18
DM, n (%)	52 (30.0)	9 (25.7)	44 (31.9)	0.35
HL, n (%)	67 (38.7)	11 (31.4)	59 (42.8)	0.25
Beta-blocker, n (%)	26 (15.0)	8 (22.9)	18 (13.0)	0.18
ARB/ACE-I, n (%)	110 (63.5)	24 (68.6)	76 (55.1)	0.18
Statin, n (%)	31 (17.9)	3 (8.6)	28 (20.3)	0.14
Calcium channel blocker, n (%)	83 (47.9)	10 (28.6)	73 (52.9)	0.01
Nitrate, n (%)	110 (63.5)	25 (71.4)	85 (61.6)	0.32
Digitalis, n (%)	18 (10.4)	6 (13.6)	12 (9.3)	0.21
ICM/non-ICM, n (%)	107 (61.8)/66 (38.1)	18 (51.4)/17 (48.6)	89 (64.5)/49 (35.5)	0.17
MMP-9, ng/mL ^a	21.1 [15.0–39.3]	28.0 [17.4–50.5]	20.0 [14.3–33.6]	<0.01
TIMP-1, ng/mL ^a	122.7 [48.6–165.1]	138.0 [49.6–182.9]	116.5 [45.7–161.2]	0.02
MMP-9/TIMP-1 ratio ^a	0.198 [0.118–0.385]	0.343 [0.135–0.553]	0.177 [0.115–0.334]	0.14
ANP, pg/mL ^a	42.2 [20.4–105.0]	73.5 [31.9–115.0]	38.7 [18.8–92.0]	0.08
BNP, pg/mL ^a	112.0 [41.9–353.5]	225.0 [44.0–620.2]	101.0 [41.9–250.5]	0.08
NA, ng/mL ^a	0.29 [0.17–0.37]	0.29 [0.20–0.92]	0.30 [0.14–0.36]	0.12
IL-6, pg/mL ^a	8.7 [4.4–14.6]	8.8 [5.3–19.4]	7.5 [4.4–14.4]	0.02
TNF-alpha, pg/mL ^a	4.2 [1.7–17.9]	11.6 [3.4–18.7]	3.1 [1.3–7.9]	<0.01
EF, % ^a	48.6 [30.2–64.1]	40.3 [17.2–55.9]	50.9 [34.3–65.1]	<0.01

ACE-I, angiotensin converting enzyme inhibitor; ANP, atrial natriuretic peptide; ARB, angiotensin II receptor blocker; BNP, brain natriuretic peptide; DM, diabetes mellitus; EF, ejection fraction; HF, heart failure; HL, hyperlipidemia; ICM, ischemic cardiomyopathy; IL, interleukin; MMP, matrix metalloproteinase; NA, noradrenaline; TIMP, tissue inhibitor of MMP; TNF, tumor necrosis factor.

^amedian value (interquartile range).

NYHA functional class and MMP molecules

Sixty-nine patients (39.8%), 73 patients (42.1%), 23 patients (13.2%), and 8 patients (4.6%) were classified as NYHA class I, II, III, and IV, respectively. Values of MMP-9, TIMP-1, and the MMP-9/TIMP-1 ratio were positively related to the NYHA functional class (*Figure 1*).

Correlations between MMP-9 and neurohormonal factors

Correlations between MMP-9 levels and various factors, including neurohormonal factors and other biomarkers, are presented in Supporting Information *Table S1*. MMP-9 levels showed weak but significantly positive correlations with TIMP-1 ($r_s = 0.395$; $P < 0.001$), NA ($r_s = 0.159$; $P = 0.036$), IL-6 ($r_s = 0.184$; $P = 0.015$), and TNF-alpha ($r_s = 0.248$; $P = 0.001$). No correlation was found between the MMP-9 level and BNP value ($r_s = 0.071$; $P = 0.35$).

Receiver operating characteristic analysis for HF events

Receiver operating characteristic analyses were performed to determine optimal cut-off values for MMP-9, TIMP-1, IL-6, BNP, NA, TNF-alpha, and the ejection fraction to detect changes in HF events. The area under the curve

of MMP-9 levels was 0.63. BNP had the highest area under the curve value among the analysed variables. For prediction of HF events, the optimal cut-off value of MMP-9 was 23.2 ng/mL, with a sensitivity of 64.7% and a specificity of 56.6%. Results of ROC analysis of other variables are shown in *Table 2*.

Hazard ratios for HF events when incorporating MMP-9 and brain natriuretic peptide levels

In the setting of both of MMP-9 and BNP levels, high MMP-9 levels (>23.2 ng/mL) and high BNP levels (>210 pg/mL) showed the highest HR for HF events (HR, 6.46; 95% CI 2.40–17.43; $P < 0.001$). Moreover, even in the lower BNP group (≤ 210 pg/mL), the HR for high MMP-9 values (>23.2 ng/mL) was 3.63 (95% CI, 1.20–11.02, $P = 0.023$) for HF events compared with low BNP values (≤ 210 pg/mL) and low MMP-9 values (≤ 23.2 ng/mL) (*Figure 2*).

Kaplan–Meier analysis for HF events

Kaplan–Meier analysis demonstrated a higher probability of HF events in patients with high MMP-9 values (>23.2 ng/mL; $P = 0.005$). Consideration of both MMP-9 and BNP levels further improved the risk stratification. For patients with low BNP levels (≤ 210 pg/mL), the risk of HF events was higher

Figure 1 Relationships between the NYHA classification and MMP-9, TIMP-1, and the MMP-9/TIMP-1 ratio. MMP, matrix metalloproteinase; NYHA, New York Heart Association class; TIMP, tissue inhibitor of MMP.

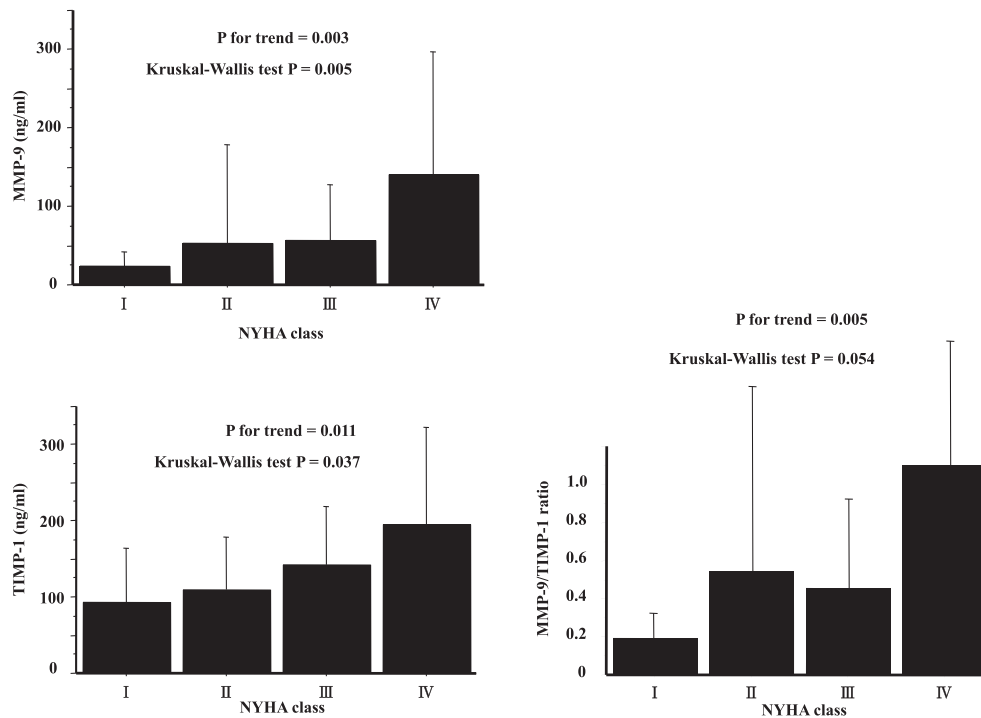


Table 2 Receiver operating curve analysis for HF events

	MMP-9, ng/mL	TIMP-1, ng/mL	BNP, pg/mL	NA, ng/mL	TNF-alpha, pg/mL	IL-6, pg/mL	EF, %
AUC	0.63	0.62	0.75	0.64	0.60	0.62	0.76
Cut-off	23.2	171.5	210.0	0.38	0.73	7.5	47.4
Sensitivity (%)	64.7	44.4	71.4	46.7	31.0	37.1	71.9
Specificity (%)	56.6	78.6	75.0	82.2	91.0	81.2	69.2

AUC, area under the curve; BNP, brain natriuretic peptide; HF, heart failure; MMP, matrix metalloproteinase; NA, noradrenaline; TIMP, tissue inhibitor of MMP; TNF, tumor necrosis factor.

for those with higher MMP-9 levels (>23.2 ng/mL; log rank $P = 0.016$) (Figure 3).

Cox proportional hazard analysis for HF events

Table 3 shows results of univariate and multivariate Cox proportional hazard model analysis for detecting HF events. In the univariate analysis, MMP-9, IL-6, ejection fraction, and BNP were predictive for HF events. The multivariate Cox proportional hazard model showed that high levels of MMP-9

were an independent predictor for HF events (HR, 3.73; 95% CI, 1.03–13.46; $P = 0.043$).

Characteristics of patients categorized by MMP-9 level

The demographics and clinical characteristics were compared between patients who had an MMP-9 level >23.2 ng/mL and those with a level ≤ 23.2 ng/mL. No significant differences were found between these groups except for MMP family members (see Supporting Information, Table S2).

Figure 2 Hazard ratios for heart failure (HF) events based on combinations of MMP-9 and BNP levels. Low MMP-9, ≤ 23.2 ng/mL; high MMP-9, > 23.2 ng/mL; low BNP, ≤ 210 pg/mL; high BNP, > 210 pg/mL. BNP, brain natriuretic peptide; CI, confidence interval; MMP, matrix metalloproteinase; Ref, reference.

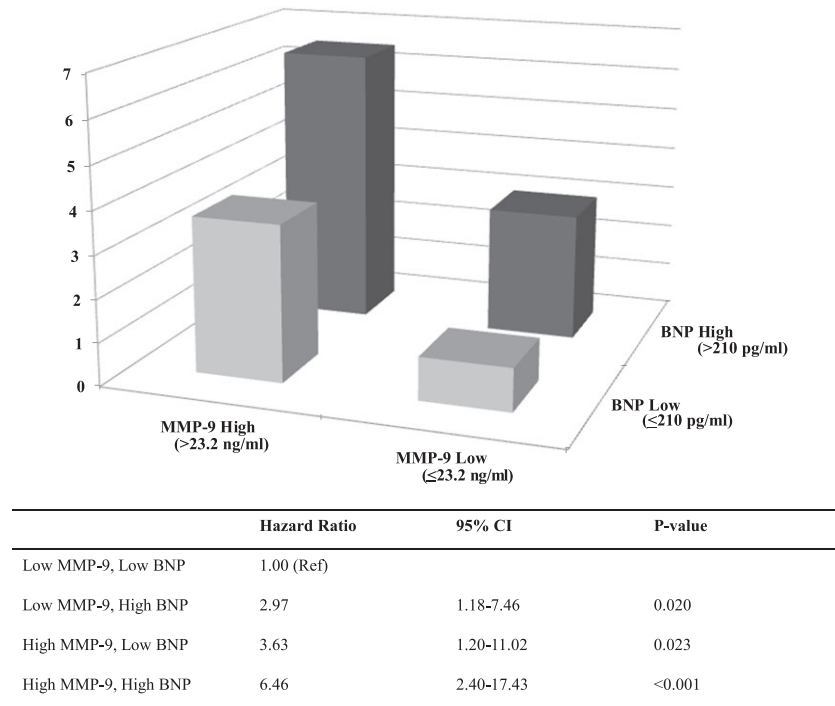


Figure 3 Kaplan–Meier curves for HF events. Upper panel: All patients were stratified by MMP-9. MMP-9 ≤ 23.2 ng/mL (solid line) vs. MMP-9 > 23.2 ng/mL (broken line). Lower left panel: Patients in the high BNP group (BNP > 210 pg/mL) were stratified into a low MMP-9 group (≤ 23.2 ng/mL) and a high MMP-9 group (> 23.2 ng/mL) (broken line). Lower right panel: Patients in the low BNP group (≤ 210 pg/mL) were stratified into a low MMP-9 group (≤ 23.2 ng/mL) (solid line) and a high MMP-9 group (> 23.2 ng/mL) (broken line). BNP, brain natriuretic peptide; HF events, heart failure events; MMP, matrix metalloproteinase.

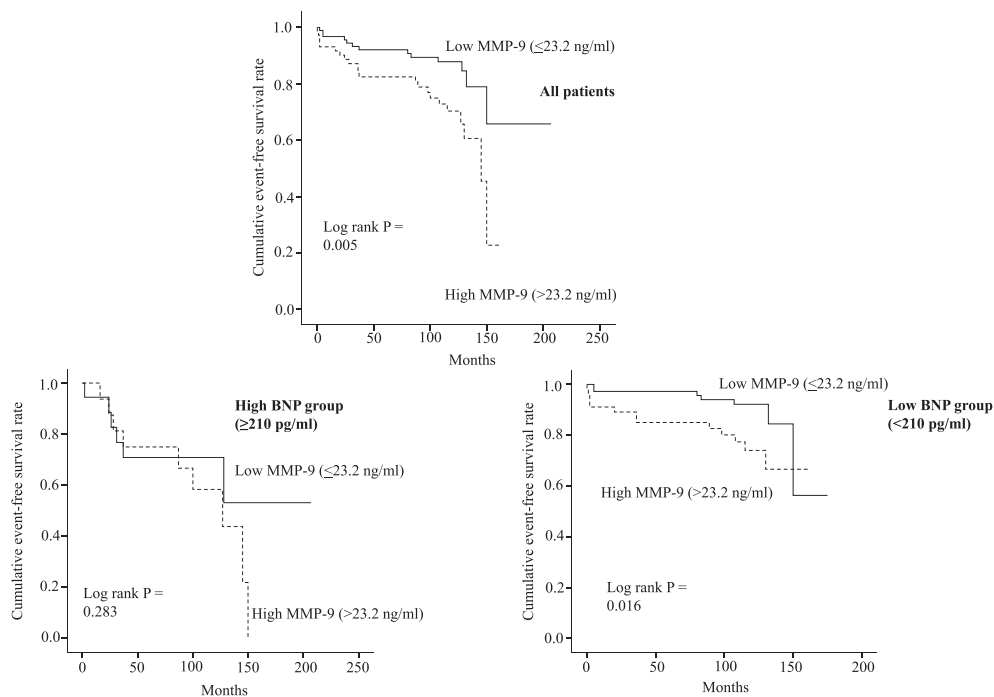


Table 3 Cox proportional hazard analysis for HF events

	Univariate HR (95% CI)	P value	Multivariate HR (95% CI)	P value
Age (years)	1.00 (0.97–1.03)	0.78	Not selected	
Sex (male)	0.90 (0.45–1.80)	0.77	Not selected	
MMP-9 (>23.2 ng/mL)	2.57 (1.29–5.12)	0.007	3.73 (1.03–13.46)	0.043
TIMP-1 (>171.5 ng/mL)	2.02 (0.97–4.20)	0.05	Not selected	
Taking a CCB	0.48 (0.23–1.01)	0.05	Not selected	
BNP (>210 pg/mL)	2.83 (1.43–5.62)	0.025	Not selected	
NA (>0.38 ng/mL)	2.44 (0.87–6.81)	0.08	Not selected	
IL-6 (>7.5 pg/mL)	2.47 (1.25–4.86)	0.009	Not selected	
TNF-alpha (>0.73 pg/mL)	1.21 (0.56–2.58)	0.62	Not selected	
EF (>47.4%)	3.50 (1.59–7.67)	0.002	Not selected	

BNP, brain natriuretic peptide; CCB, calcium channel blocker; CI, confidence interval; HF, heart failure; HR, hazard ratio; MMP, matrix metalloproteinase; NA, noradrenaline; TIMP, tissue inhibitor of MMP; TNF, tumor necrosis factor.

Additive information of MMP-9 to brain natriuretic peptide

Incorporating MMP-9 into BNP yielded a significant category-free NRI of 0.291 (95% CI 0.015 to 0.567) and IDI of 0.055 (95% CI 0.018 to 0.093), these findings were statistically significant.

Discussion

The main findings of the present study are: first, in patients with chronic HF, MMP-9, TIMP-1, and the MMP-9/TIMP-1 ratio were correlated with disease severity as determined by the NYHA functional class. Second, MMP-9 values were correlated with inflammatory cytokines and neurohormonal factors in patients with chronic HF. Third, even in patients with low BNP levels, high MMP-9 levels were a strong predictor of HF events in a long-term follow-up of a median of 109 months. Fourth, reclassification metrics such as NRI and IDI were statistically improved on incorporation of the MMP-9 level, the additive clinical usefulness of MMP-9 to BNP was shown.

We demonstrated the additive prognostic value of considering both MMP-9 and BNP levels. Several potential reasons may explain our observations. BNP-guided therapy does not always improve clinical outcomes as previously reported.^{9–11} The reason for this lack of significant improvement may be that BNP levels only change upon ventricular wall stretching. Thus, worsening of HF must occur before BNP levels rise. Elevated MMP-9 levels may help identify patients at risk before an increase occurs in ventricular pressure overload, which reflects ongoing ventricular remodeling. The value of BNP levels for guiding therapy in addition to clinical symptom-based treatment seems to be limited,^{9–11,21} despite

the undisputed diagnostic and prognostic importance of these values.^{6–8} The benefits of predicting HF events may be offset by non-HF events. Although BNP measurement can help detect worsening HF, the current standard HF therapy is not sufficient to prevent subsequent HF events. Because deterioration of heart function must occur before BNP levels rise, elevated levels of another biomarker before an increase in cardiac pressure occurs may help identify patients at risk for HF events. At such an early phase, medical interventions can prevent a poor outcome.

BNP is a cardiac loading marker that responds to ventricular and myocardial stretching and wall stress, whereas MMP is regarded as a marker of fibrosis and is less responsive to loading. Our study demonstrated that in HFPEF patients, levels of BNP, and the MMP-9/TIMP-1 ratio were lower compared with those in HFREF patients. An imbalance in the MMP/TIMP ratio and a robust increase in BNP levels reflect advanced ventricular remodeling, dilatation, and wall stretching. MMP and TIMP levels were similar in HFREF and HFPEF patients and may represent ongoing myocardial injury and extracellular matrix remodeling before an increase in BNP and a decreased ejection fraction are seen. HFPEF is characterized by matrix apposition and myocardial stiffening. Thus, a matrix and fibrosis marker such as MMP may also be an important prognostic marker in HFPEF. We focused on MMP-9, TIMP-1, and the MMP-9/TIMP-1 ratio as candidate markers for predicting HF events in this study. No significant change was observed in the MMP-9/TIMP-1 ratio in patients with and without HF events. TIMPs have been proposed as potential biomarkers of HF development. However, in our study, the combination of MMP/TIMP levels could not predict HF events during the follow-up period. These observations imply that only measuring MMP-9 and not TIMP-1 may be sufficient to assess the risk of future HF events.

MMP molecules are up-regulated in non-structural remodeling situations, such as lethal arrhythmia.^{22,23} The HR for HF events was highest in conditions of high BNP and high MMP-9 levels. These conditions appear to reflect the status of ongoing inflammation and catecholamine spillover in addition to ventricular wall stretching. Thus, the combination of both high BNP and high MMP-9 levels was associated with a poor outcome in HF patients. Notably, high MMP-9 levels even in the presence of low BNP levels still represented a high clinical risk for HF events (HR 3.63). This situation represents ongoing ventricular remodeling via inflammation and catecholamine spillover before a ventricular pressure overload occurs. Indeed, we observed significant correlations between MMP-9 and factors such as IL-6, NA, and TNF- α levels, whereas no correlation was found between MMP-9 and BNP levels. We therefore consider that elevated MMP-9 levels are a prognostic factor for a worsening clinical course, regardless of BNP levels.

The addition of MMP-9-guided therapy to BNP-guided therapy leads to additional risk stratification, which allows further detection of patients at risk of HF events and allows more aggressive drug and/or mechanical intervention to avoid HF events. Compared with BNP monitoring, MMP-9 monitoring was associated with significantly better prediction of worsening HF events. Cox hazard analysis demonstrated that MMP-9 was still prognostic in the setting of low BNP. In this condition, although extracellular matrix remodeling and turnover were accelerated, ventricular wall stress was not increased enough to stimulate a robust increase in BNP. Hence, MMP-9 monitoring enables identification of patients with a risk of worsening HF events at an earlier time compared with BNP monitoring. In this study, we could not clarify the determinant of differences in plasma MMP-9 levels. However, several interventions attenuate MMP activity^{24–30}; thus, assessing MMP levels is useful for identifying patients at risk for HF events and could be used to monitor patients who may require modification of their therapeutic options. Increased MMP-9 may identify a subset of HF patients who would benefit from intensive medical therapy. Risk stratification may be best achieved by using models that incorporate multiple biomarkers, including inflammatory cytokines and natriuretic peptides. Associations between adverse left ventricular remodeling and worse prognosis, and MMPs have been investigated in various HF conditions and subject's background.^{31–33} Our study showed MMP-9 levels are a strong predictor of HF events in patients with chronic HF. Our findings go line with recent findings on the impact of MMP-9 in patient with HF, while there are inconsistent findings regarding TIMP-1 levels. This discordance may reflect the changes of MMP species expressions in various stages and conditions of HF in each study. For cardiac pressure overload, there are increased levels of TIMPs in the early stages of HF associated with extracellular matrix accumulation. In the late phase, such as

left ventricular dilatation, there is an up-regulation of MMPs leading to extracellular degradation.^{1,34,35} Up-regulations of both MMP-9 and TIMP-1 in patients with poor prognosis seems to reflect varieties of ventricular remodeling stages at the time of assessment.

Our study has several limitations. This study consisted of observational research in patients already diagnosed with HF, and hence did not establish a cause-effect relationship of interventional therapy. We also did not examine individuals without HF. ROC analyses showed relatively low areas under the curves for all variables. ROC analyses are suitable for events that are currently happening, rather than predicting events for long-term follow-up periods. Another limitation is that MMP molecules are up-regulated in non-HF events such as unstable angina, myocardial infarction, and cerebral infarction. Thus, specificity and the area under the curve were relatively low. To predict major adverse cerebro-cardiovascular events, MMP-9, and BNP were still strong predictors (data not shown). The proportion of patients receiving beta-blockers was low at the time of blood collection. In the era of enrollment, calcium channel antagonists were widely used to treat hypertension and angina pectoris, particularly in Japan³⁶ where coronary artery spasm is more prevalent than in the West.³⁷ Beta-blockers are thought to aggravate coronary spasm.³⁸ After enrollment, patients received tailored medical therapy by the in-charge cardiologists. Tailored HF therapy may have affected the incidence of HF events. However, in-charge cardiologists and patients were blinded to the results of biomarkers, except for BNP and ejection fraction values. Thus, we consider that baseline MMP-9 values are still predictive regardless of tailored therapy. In HF conditions, the majority of BNP is produced from ventricular myocardium. BNP is synthesized as preprohormones and cleaved to prohormones, proBNP_{1–108}, that are proteolytically cleaved to bioactive form, BNP_{1–32}.^{39,40} Conventional assay for BNP, which was available in our study, are not specific because antibody against BNP_{1–32} has cross-activity with proBNP_{1–108}. Our measurement of BNP recognized both proBNP_{1–108} and BNP_{1–32}. Therefore, novel, specific assay for proBNP_{1–108} may establish additional predictive ability for progressive HF events. The incidence of cardiac events was substantially lower than that reported in western countries.^{36,41} Racial or ethnic differences between Japanese and western populations may mediate a difference in the response to HF therapy. However, the possibility that the low event rate may be due to the selection of low-risk patients cannot be excluded. Over-activation of MMPs, or an imbalance of active MMPs and their inhibitor TIMPs, is considered to be a key factor in the remodeling of extracellular matrix found in HF conditions. It is therefore of interest to determine not only the amount of enzyme expressed, but also what proportion subsequently becomes activated or has the ability to become active. However, ELISA system, which was

used in our study, detects proMMP-9 and their complex form with TIMP-1.¹⁸ Thus, our study may not reflect the actual ratio of MMP-9: TIMP-1. This may lead to underestimate the competence of predictive ability of MMP-9/TIMP-1 ratio.

In conclusion, MMP-9 levels and the level of inflammatory cytokines were correlated with the severity of chronic HF. MMP-9 levels are a strong predictor of HF events and allow further risk stratification of patients, suggesting that disparity between MMP-9 and TIMP-1 levels may contribute to HF events and mortality.

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Conflict of interest

None declared.

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Supporting information

Supporting information may be found in the online version of this article.

Table S1. Correlations with MMP-9 levels.

Table S2 Patient characteristics by MMP-9 value.

Figure S1. Bar graphs illustrating MMP-9, TIMP-1, the MMP-9/TIMP-1 ratio, and BNP levels in HFREF and HFPEF patients.

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