Published in final edited form as:

Int J Cardiol. 2011 September 1; 151(2): 237–239. doi:10.1016/j.ijcard.2011.06.048.

Matrix Metalloproteinases, Tissue Inhibitors of Metalloproteinases, and Heart Failure Outcomes

Vikas Bhalla, MD¹, Vasiliki V. Georgiopoulos, MD¹, Andreas P. Kalogeropoulos, MD¹, Catherine P. Norton, MD¹, Robert T. Cole, MD¹, Sonjoy R. Laskar, MD¹, Catherine DeStaerake, PhD², W. Craig Hooper, MD², Andrew L. Smith, MD¹, and Javed Butler, MD MPH¹

¹Emory University, Atlanta, GA

²Centers for Disease Control and Prevention, Atlanta, GA

Heart failure (HF) prevalence continues to increase and is associated with a high mortality, morbidity, and cost burden for the society. [1, 2] The most common cause of HF in the United States is coronary artery disease. [3] Left ventricular (LV) dysfunction, irrespective of the cause of heart failure (HF), leads to perturbed wall stress resulting in remodeling and HF progression. Matrix metalloproteinases (MMP) are a family of proteolytic enzymes that are involved in the protein degradation in the extracellular matrix and play an important role in remodeling. [4, 5] Multiple classes of MMPs have been identified in human myocardium. [5, 6] Certain MMPs, specifically MMP-2, MMP-3, and MMP-9 have been shown to have high expression in the left ventricle. [7, 8] Tissue inhibitors of metalloproteinases (TIMPs) are low-molecular-weight molecules that bind to MMPs forming MMP-TIMP complexes that exhibit an inhibitory control on MMPs. Several studies have delineated the relationship between MMPs and TIMPs, and that the changes in the MMP/TIMP ratio correlate with left ventricular hypertrophy and dilation. [9, 10] Loss of inhibitory control of TIMP on MMP correlates with progression of left ventricular remodeling via increased MMP activity, extracellular matrix proteolysis, and myocardial remodeling changes. [11] There have been conflicting reports on MMPs and TIMPs levels association with HF outcomes. [12-17] These studies have generally not assessed the multiple members of the MMP and TIMP family simultaneously and have assessed varying HF outcomes. In this study, we sought to assess the association between multiple MMPs and TIMPs with clinical outcomes, health status, and exercise capacity among HF patients.

We examined the association of baseline serum levels of MMP 1, 2, and 9 and TIMP 1, 2, 3, and 4 with outcomes among 147 outpatients with HF from 1/2008 to 7/2009 enrolled in the Atlanta Cardiomyopathy Consortium, a prospective cohort study enrolling HF patients from three university-affiliated teaching hospitals. All patients undergo detailed history surveys, electrocardiogram, six minute walk test, standardized questionnaires, and collection of blood and urine samples. Every six months, the patients are contacted to assess outcomes including

Address correspondence to: Javed Butler, MD MPH, Emory University Hospital, Center for Heart Failure Therapy, 1365 Clifton Road NE, Suite AT 430, Atlanta, GA 30322. Telephone No: 404-778-5273 Fax No: 404-778-5285 javed.butler@emory.edu.

Conflict of interest: None

medication changes, procedures, new diagnoses, and hospitalizations. Mortality data are collected through medical record review, information obtained from family members, and Social Security Death Index query. Hospitalization data are obtained from regular electronic health records review, all outpatient notes from any specialty encounter for any reported admission to an outside hospital, and direct patient inquiry during follow-up.

Levels of MMPs and TIMPs were measured by Fluorokine MAP Human MMP and TIMP kits with a coefficient of variation < 10% for intra-assay and <15% for inter-assay testing.

Clinical outcome was defined as a composite of death, cardiac transplantation, or left ventricular assist device placement or HF hospitalizations. Exercise capacity was determined using the six minute walk test. [18] Health status was assessed with the Kansas City Cardiomyopathy Questionnaire (KCCQ), a 23-item tool with scales that measure multiple aspects of health status in HF patients including clinical symptoms, self-efficacy, physical limitation, social limitation and quality of life. [19] A difference of five points on the KCCQ scale scores is considered clinically important. [20]

Correlations between biomarkers, ejection fraction, and exercise capacity were assessed with Pearson correlation. Outcomes were described with Kaplan–Meier estimates and compared with the log-rank statistic between groups. The association of biomarkers with outcomes was examined with Cox proportional hazards models. Patients were divided into two groups based on linearity of strongest associated biomarker with outcomes. Other continuous non time dependent variables in these groups were compared with t-test. Multivariable Cox proportional hazards analysis was then performed adjusting for independent predictors of outcomes in univariate analysis (p-value <0.20). Statistical analysis was done using software from SAS Institute Inc., Cary, Version 9.2 NC, USA.

Table 1 shows baseline characteristics. Mean age of patients was 57.1 ± 11.9 years, 67% were male; and 58% were white. Patients were on optimal medical therapy. Mean ejection fraction was 24.6 ± 10.9 % and mean BNP level was 510 ± 720 pg/ml. During follow-up of 23.1 ± 6.7 months, 49 patients (33%) experienced clinical outcome (22 death or transplant or LVAD placement and 27 HF hospitalizations). Among the biomarkers, there were several inter-correlations; MMP-2 and TIMP-2 had the strongest correlation ($r = 0.77$, $p < .0001$) (**Table 2**).

In univariate Cox regression analysis TIMP-2 was the strongest predictor of the outcome among the biomarkers of collagen turnover (hazard ratio [HR] 1.019, 95% confidence interval [CI] 1.007, 1.032 for each 1 pg/ml increase) (**Table 3**). Univariate predictors of events included, ejection fraction (HR 0.96, CI 0.931-0.989), BNP (HR 1.001 CI 1.00 - 1.001) and creatinine (HR 1.27 CI 1.15-1.4). Age, Gender and race were not significant predictors of adverse events. History of smoking (43% vs. 26%, $p = .001$) and sleep apnea (63% vs. 26%, $p = .03$) were significant predictors of outcomes whereas diabetes had a strong trend (42.8% VS. 19.5%, $P = 0.08$).

TIMP-2 levels were dichotomized at fourth quartile (133.82 pg/ml) based on linearity of events (1st quartile= <108.7 pg/ml, event rate 18.9%; 2nd quartile= $121.15-108.7$ event rate 16.67%; 3rd quartile= $133.82-121.5$, event rate 38.16%; and 4th quartile= >133.82 , event

rate 59.5%). TIMP-2 was negatively correlated to ejection fraction ($r = -0.26$, $p=0.002$) and six minute walk test ($r = -0.19$, $P=0.03$); and positively with BNP ($r=0.20$, $p=0.03$). The group with value >133.82 pg/ml had significant lower ejection fraction (19.3% vs. 26.3%, $p<0.001$), less distance covered during the six minute walk test (335 meters vs. 382 meters, $p<0.016$) and lower KCCQ physical limitation score (61.6 vs. 71.6, $p<0.024$). Overall KCCQ scores were not different (67.5 vs. 62.2, $p = 0.19$)

In Cox regression model controlling for age, race, gender, ejection fraction, smoking, sleep apnea, diabetes, depression, BNP, creatinine, and baseline therapy, TIMP-2 level >133.82 was an independent predictor of outcomes (HR 2.89 95% CI 1.09 , 7.7) **Figure 1**.

Prior studies have shown the prognostic utility of MMP-1 [12] ,MMP-2[13] , and TIMP-1 [14] in HF. MMP-1 is shown to correlate with functional capacity [12] [15], neurohormonal activation[16], and BNP levels [17]. However, these and other studies have shown conflicting results. [12] [15] [13] [14] [21]. Our study is unique since we compared several MMPs and TIMPs for multiple outcomes. We found that highest TIMP-2 quartile has thrice the event rate when compared with the lowest quartile. We also found that TIMP-2 negatively correlate with Physical Limitation Score.

The important question raised from our data is that what makes TIMP-2 the best predictor among the biomarkers of collagen turnover. It is generally accepted that MMPs are harmful and TIMPs are beneficial for extracellular matrix and lower TIMP levels could be detrimental in HF. The review by Brew and Nagase points to several independent effects of TIMP-2 including anti-angiogenicity [22]. We can only hypothesize that maybe this is what we are observing in our data. Myocardial remodeling is the central culprit in the progression of chronic systolic HF [23] and therapies that inhibit myocardial remodeling [24, 25], such as angiotensin-converting enzyme inhibitors, β -blockers and aldosterone antagonists help ameliorate clinical outcomes including HF hospitalization and death[26]. Whether TIMP-2 levels have the potential of accurately guide therapy and help optimize this management needs study. Our limitation was that our study was an observational cohort study with a smaller sample size. Also, we do not have the data on changes in biomarker levels over time.

We conclude that elevated serumlevels of TIMP-2 levels correlate with MMP-2, BNP, creatinine levels, ejection fraction, quality of life indices and functional capacity; and were strongly and independently associated with adverse outcomes in this cohort of outpatients with primarily systolic HF. Further experimental studies are needed to explore the utility of TIMP-2 to help guide management and therapy in heart failure patients.

Acknowledgments

Funding source: This project was partially funded by the PHS Grant (UL1 RR025008, KL2 RR025009 or TL1 RR025010) from the Clinical and Translational Science Award program, National Institutes of Health, National Center for Research Resources.

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Figure 1.
Kaplan-Meier survival curve of the two groups divide at TIMP-2 >133.82 pg/ml

Table 1

Study population baseline characteristics.

Characteristic	%
Female	33%
White	58%
History of smoking	27%
Ischemic Etiology	33%
Systolic heart failure	99%
Diabetes	33%
Hypertension	64%
Dyslipidemia	51%
Chronic kidney disease	31%
Peripheral arterial disease	2.5%
Sleep apnea	20%
Depression	17%
Beta-blocker	94%
ACE inhibitor or angiotensin receptor blocker	84%
Statins	53%
Implantable cardioverter defibrillator and/or biventricular pacemaker	73%
Coronary artery bypass graft, %	19%
Age (years)	57±12
BNP (ng/ml) (N=117)	510±720
Creatinine (mg/dl)	1.42 ±1.35
MIMP-1, (ng/ml)	3373±2695
MMP-2, (ng/ml)	255±79.5
MMP-9, (ng/ml)	246.6±128.1
TIMP-1, (pg/ml)	143.5±140.9
TIMP-2, (pg/ml)	124±21.8
TIMP-3, (pg/ml)	16.8±11.4
TIMP-4, (pg/ml)	1829±1071.6

Table 2

Correlation among biomarkers of collagen turnover

Biomarker		MMP-2	MMP-9	TIMP-1	TIMP-2	TIMP-3	TIMP-4
MMP-1	r	0.02	0.16	0.05	0.07	0.12	0.10
	p	0.80	0.06	0.56	0.40	0.16	0.22
	n	147	147	147	147	147	147
MMP-2	r		−0.24	0.11	0.77	0.09	0.08
	p		0.004	0.20	<.0001	0.30	0.33
	n		147	147	147	147	147
MMP-9	r			0.00	−0.06	0.10	−0.09
	p			0.97	0.44	0.25	0.30
	n			147	147	147	147
TIMP-1	r				0.27	0.17	0.18
	p				0.001	0.04	0.03
	n				147	147	147
TIMP-2	r					0.23	0.21
	p					0.005	0.01
	n					147	147
TIMP-3	r						0.38
	p						<.0001
	n						147