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

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

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An abstract of

A thesis submitted to the Faculty of

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in partial fulfillment of the requirements for the degree of

Master of Science in Clinical Research 2011

ABSTRACT

Background: Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are involved in cardiac remodeling through regulation of extracellular matrix. Reports on their association with heart failure (HF) outcomes have been conflicting. **Methods:** We prospectively examined the association of baseline serum levels of MMP 1, 2, and 9, and TIMP 1, 2, 3, and 4, with; a) combined outcomes (death or cardiac transplantation or left ventricular assist device implantation), b) HF hospitalization, c) six minute walk distance, and d) KCCQ Scores; in 147 ambulatory HF patients. Levels of MMPs and TIMPs were measured by Fluorokine MAP Human MMP and TIMP Kits.

Results: Mean age of patients was 57.1± 11.9 years, 67% were male; and 58% were white. Mean ejection fraction (EF) was 24.6 ± 10.9 %. During follow-up of mean 23.1 ± 6.7 months, 49 patients (33%) experienced combined outcome. 22 of these 49 patients had either died, had transplants or LVADS and remaining 27 had HF related hospitalizations . Among the biomarkers of collagen turnover, there were several inter-correlations but MMP-2 and TIMP-2 had the strongest correlation to each other(r= 0.77, p=<.0001). As for cardiac structural and functional parameters, TIMP-2 was significantly and negatively correlated to EF (r = -0.26, p=0.002, n=146) and Six Minute Walk Test(r= -0.19, P=0.03, n=137). TIMP-2 was also positively and significantly correlated to cardiac neurohormone BNP (r=0.20, p=0.03, n=117). In Univariate Cox regression analysis TIMP-2 was the strongest predictor of the outcome among the biomarkers of collagen turnover (HR 1.019, 95%CI(1.007 -1.032) for each 1 pg/ml increase. When dichotomized at 133.82 pg/ml based on linearity, the event rate in the group > 133.82 pg/ml was 59.5% n=37 vs < 133.82 pg/ml was 24.5 % n=110.

In Cox regression model controlling for age, gender, EF, past medical history, creatinine, and current therapy, TIMP-2 level > 133.82 was independent predictor of adverse outcomes (OR 6, 95%CI 2.22-16.12). The final parsimonious multivariate cox regression Model containing TIMP-2, age, creatinine and sleep apnea, TIMP-2 level > 133.82 had a HR of 4.46(2.1-9.4).

Conclusion: Elevated serum levels of TIMP-2 were independently associated with adverse outcomes in HF.

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Introduction and Background

Heart failure (HF) prevalence continues to increase and is associated with a very 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, extra-cellular 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 cardiac structure and function, exercise capacity, quality of life, and outcomes among HF patients.

METHODS

We examined the association of baseline serum levels of MMP 1, 2, and 9 and TIMP 1, 2, 3, and 4 with outcomes (death, cardiac transplantation, left ventricular assist device implantation, or HF hospitalization) in 147 stable outpatients with HF who were enrolled in a prospective HF cohort study in the greater metropolitan Atlanta, GA area from 1/2008 to 7/2009.

Design:

Prospective Observational Cohort Study.

Hypotheses:

1. Biomarkers of collagen turnover will correlate strongly among themselves, with cardiac structure/function and functional capacity in HF patients.

2. Biomarkers of collagen synthesis will independently predict outcomes in HF patients and add to risk stratification.

Patient Population:

The Atlanta Cardiomyopathy Consortium is a prospective cohort study enrolling HF patients from three university-affiliated teaching hospitals in the greater metropolitan Atlanta area. All patients undergo detailed medical history surveys, electrocardiogram, six minute walk test, standardized questionnaires, and collection of blood and urine samples at baseline. Race/ethnicity is self-reported as is education level, as number of school years completed. Every six months, the patients are contacted to assess outcomes including 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. At the time of this analysis, a total of 147 patients were enrolled in this study.

Selection Criteria:

Inclusion criteria

Male or female greater than 18 years of age

Able to understand and sign written informed consent

Willingness to participate in required follow-up exams

Diagnosis of heart failure

Exclusion criteria

Congenital heart disease

Previous heart transplantation or on current waiting list

Valvular heart disease

Uncontrolled blood pressure greater than 140/90

Biomarkers:

Levels of MMPs and TIMPs were measured by Fluorokine MAP Human MMP and TIMP Kits. As per Manufacturer's label: all MMPs and TIMPS ELISA tests have a coefficient of variation value (CV %) < 10% for intra-essay testing and <15% for inter-Essays testing.

Outcomes:

Outcome was defined as a composite of death, cardiac transplantation, or left ventricular assist device placement and HF hospitalizations. Exercise capacity was determined using the 6-minute walk test, a simple measure of functional capacity in HF patients. [18] Health status was assessed with the Kansas City Cardiomyopathy Questionnaire (KCCQ), a 23-item self-administered 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] The KCCQ has been established as a valid, reliable, and responsive health status measure for HF patients. [20] The KCCQ scales are summarized into a single summary score ranging from 0-100 reflecting overall health status, with higher scores reflecting better functioning, fewer symptoms, and better quality of life. A difference of five points on the KCCQ scale scores is considered clinically important. [21]

Statistical Analysis:

Data was expressed in mean ± stdev and 95% confidence intervals were displayed as appropriate. Correlations between biomarkers, echocardiographic, and exercise parameters were performed with the use of Pearson correlation. For outcomes assessment, for categorical variables , univariate analysis was done using Kaplan-Meier method and compared with the log-rank statistic, for continuous variables, univariate Cox proportional hazards analysis was performed to find significant relationships of events with biomarkers. Patients were ultimately divided into two groups based on linearity of strongest associated biomarker among the markers of collagen turnover and their survival was compared using Kaplan-Meier method. Other continuous non time dependent variables in these groups were compared with ttest. Multivariable Cox proportional hazards analysis was then performed adjusting for independent predictors of outcomes in univariate analysis (P-value of <0.2) and conventional predictors of outcomes including age, gender, ejection fraction, past medical history, creatinine, and current therapy. A best fit yet parsimonious model was selected using the likelihood ratio test. Multicollinearity and interactions were tested in the Cox regression model. The proportional hazard assumption was tested using the graph of the log(-log(survival)) versus log of survival time graph. Example KM survival curves were plotted for individuals (with model covariates) to display the outcomes. Model was validated using C-statistic. Statistical analysis was done using software from SAS Institute Inc., Cary, NC, USA.

RESULTS

Mean age of patients in the cohort was 57.1± 11.9 years, 67% were male; and 58% were white, 99% had systolic dysfunction, 33% had diabetes, 64% hypertension and 20% had sleep apnea. Thirty three percent of the cohort had heart failure from ischemic etiology. **Table 1a** shows study population baseline characteristics. As for the treatment, 94% were on beta-blockers, 84% were on either ACE inhibitor or angiotensin receptor blocker and 73% had Implantable cardioverter defibrillator and/or biventricular pacemaker. **Table 1b** shows the treatment and management distribution in the cohort. Mean ejection fraction was 24.6 ± 10.9 % and mean BNP level was 510± 720 pg/ml. **Table 1c,** shows the distribution of the biomarkers in the cohort.

Outcomes:

During follow-up of mean 23.1 ± 6.7, 95% CI (22-24.4 months), 49 patients (33%) experienced combined outcome. 22 of these 49 patients had either died, had transplants or LVADS and remaining 27 had HF related hospitalizations .

Correlations:

Among the biomarkers of collagen turnover, there were several weak inter-correlations but MMP-2 and TIMP-2 had the strongest correlation to each other(r= 0.77, p=<.0001). **(Table 2a)** As for cardiac structural and functional parameters, TIMP-2 was significantly and negatively correlated to ejection fraction (r = -0.26, p=0.002, n=146) and six minute walk test(r= -0.19, P=0.03, n=137). TIMP-2 was also positively and significantly correlated to cardiac neurohormone BNP (r=0.20, p=0.03, n=117). **(Table 2b)**

Univariate analysis:

In univariate cox regression analysis TIMP-2 was the strongest predictor of the outcome among the biomarkers of collagen turnover (HR 1.019, 95%CI (1.007 -1.032) for each 1 pg/ml increase **(Table 3a)**. **Table 3b** shows univariate prediction power of age, EF, BNP and creatinine

using cox regression analysis. Further survival analysis of binary variable was done using lifetest procedure (Kaplan-meier analysis). Gender and race were not significant predictors of adverse events (**Table 3c)**. **Table 3d** shows univariate prediction power of past medical history variables. History of smoking (Event rate 43% vs 26%, p=.001) and Sleep Apnea (Event rate 63% vs 26%, p=.03) were significant predictors of outcomes.

When dichotomized at 133.82 pg/ml TIMP-2 level, based on linearity, the event rate in the group > 133.82 pg/ml was 59.5% n=37 vs < 133.82 pg/ml was 24.5 % n=110 **(Table 4a)**. The high risk group (group > 133.82 pg/ml) had significant lower EF [mean (95% CI) 19.3 (15.9 - 22.6) vs 26.3 (24.3 - 28.3) p< 0.001] significantly smaller average distant covered during the six minute walk test [mean (95% CI) 382 (363 - 401.2) vs 335 (302.3 - 368.1) p<0.016] and significantly lower KCCQ Physical limitation Score [mean (95% CI) 71.6 (67.1 - 75.99) vs 61.6 (54.9 - 68.3) p<0.024]**(Table 4b)**.

In Cox regression model controlling for age, race, gender, ejection fraction, past medical history, and creatinine, TIMP-2 level > 133.82 was independent predictor of adverse outcomes (odds ratio 6 , 95% confidence interval 2.22-16.12) (**Table 5a)**. The final best fit yet parsimonious model selected using the likelihood ratio test had TIMP-2, age, creatinine and sleep apnea, as best predictors of the outcome. [**Table 5b**, TIMP-2 > 133.82 had a HR of 4.46 (2.1-9.4)]. Interestingly we found an interaction of TIMP-2 with sleep apnea. Model when cross-validated using logistic regression yielded a C-statistic of 83.4 for training model and 79.4 for cross-validation model.

Figure 1a shows the Kaplan-Meier survival curve of the two groups divide by a cutpoint of TIMP-2 > 133.82 pg/ml. **Figure 1b** shows the example KM survival curves plotted for a 50 year old individual (with model covariates) to display the outcomes with various risk factors. Interestingly the two year survival for that 50 year old patient with > 133.82 TIMP-2 levels was less than that of a 50 year old with > 133.82 TIMP-2 and Sleep apnea.

Comparison with BNP:

After adding BNP levels to the model, TIMP-2 > 133.82 were still significant predictor of outcomes (HR 3, 95% CI (1.4 - 6.5). BNP levels did not improve the overall model (Likelihood ratio test statistic was reduced to 28.8 for model with BNP Vs 34.4 of original model) as it may not be truly independent from TIMP.

Discussion

Prior studies have shown the prognostic utility of MMP-1[12] ,MMP-2[13] , TIMP-1 [14] in HF. MMP-1 is shown to correlate with functional capacity [12] [15], neuroharmonal activation[16], and BNP [17]. However, these and other studies have shown conflicting results. [12] [15] [13] [14] [22]. Our study is unique since we compared several MMPs and TIMPs for multiple outcomes. We found that MMP2 and TIMP2 negatively correlated with EF and positively with BNP, and TIMP1 and TIMP3 correlated negatively with six minute walk test. Only TIMP-2 negatively and significantly correlates with both functional capacity and EF.

We examined the event rate and found that highest TIMP-2 quartile has thrice the event rate when compared with the lowest quartile. We used rigorous time to event analysis and developed a parsimonious model to predict outcome in this cohort. We showed individual 2 year survival curves for a 50 year old hypothetical patient with various risk factors from our model. Interestingly we found that the survival was improved in the patients with the history of sleep apnea. We hypothesize that this is from the subsequent intervention, for example non invasive positive pressure ventilation, and not the disease itself as sleep apnea is known to worsen outcomes in heart failure patients.[23] We plan to further explore this in a subsequent interventional Randomized controlled study.

We also examined the correlation of biomarkers with KCCQ quality of life measures and found that MMP-1 and 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 [24]. We can only hypothesize that maybe this is what we are observing in our data.

As we know, myocardial remodeling is the central culprit in the progression of chronic systolic HF [25]and therapies that inhibit myocardial remodeling[26, 27], such as angiotensin-converting enzyme inhibitors and β-blockers, do help ameliorate clinical outcomes including HF hospitalization and death[28]. We believe TIMP-2 is one of those biomarkers that has the potential of accurately guiding this therapy and help optimize this management. We are planning further studies to see whether the changes in TIMP-2 level overtime could be a better predictor of adverse events the one time baseline measurement. These studies could eventually become the basis for evaluating the utility of TIMP-2 biomarker guided therapy to improve outcomes in heart failure patients.

Limitations:

Our limitation was that our study was an observational prospective cohort study, and we used the baseline levels of biomarkers for prediction and for other comparisons. It is possible that a randomized trial design and the dynamic measurements (delta) from serial monitoring might give us even better results. Our limitation is also our strength as this study is conducted in real world fashion with diverse ethnicity and good gender distribution in the cohort, and hence the results could be more generalize-able.

Conclusion:

We concluded that elevated serum levels of TIMP-2 levels correlate with MMP-2 , BNP, Creatinine levels, Ejection Fraction, Quality of life indices (KCCQ) and functional capacity (six minute walk test); and were strongly and independently associated with adverse outcomes in this cohort of stable 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.

Tables and Figures

Table 1a : Study population baseline characteristics.

Characteristic	n (%)	Total N
Female	49 (33%)	147
White	85 (58%)	147
History of smoking	40 (27%)	129
Ischemic Etiology	49 (33%)	146
Systolic heart failure	145 (99%)	147
Diabetes	49 (33%)	147
Hypertension	94 (64%)	147
Dyslipidemia	75 (51%)	146
Chronic kidney disease	45 (31%)	146
Peripheral arterial disease	4 (2.5%)	146
Sleep apnea	30 (20%)	146
Depression	25 (17%)	147

Table 1b: Treatment and management distribution in the cohort.

Treatment	n (%)	Total N
Beta-blocker	138 (94%)	147
ACE inhibitor or angiotensin receptor blocker	123 (84%)	147
Statins	78(53%)	147
Implantable cardioverter defibrillator and/or biventricular pacemaker	107 (73%)	146
Coronary artery bypass graft, %	28 (19%)	146

Table 1c : Distribution of the biomarkers in the cohort.

Variable	n	Mean	Stdev	95% CI
MIMP-1, (ng/ml)	147	3373	2695	2934 – 3813
MMP-2, (ng/ml)	147	255	79.5	242 – 268
MMP-9 , (ng/ml)	147	246.6	128.1	225.7- 267.5
TIMP-1, (pg/ml)	147	143.5	140.9	120.5 – 166.5
TIMP-2 ,(pg/ml)	147	124	21.8	120.5 – 127.6
TIMP-3, (pg/ml)	147	16.8	11.4	14.9 - 18.7
TIMP-4, (pg/ml)	147	1829	1071.6	1651 – 2001
BNP (ng/ml)	117	510	720	378 – 642
Creatinine (mg/dl)	143	1.42	1.35	1.19 - 1.64

Table 2a: Correlation among biomarkers of Collagen Turnover.

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

Table 2b: Correlation of biomarkers of collagen turnover with cardiac function, functional capacity and BNP.

		MMP-1	MMP-2	MMP-9	TIMP-1	TIMP-2	TIMP-3	TIMP-4
Ejection fraction	r	-0.18	-0.29	-0.11	-0.06	-0.26	-0.04	-0.13
	p	0.03	<0.001	0.20	0.46	0.002	0.61	0.10
	n	146	146	146	146	146	146	146
Six Minute Walk Test	r	-0.04	-0.13	0.13	-0.19	-0.19	-0.03	-0.28
	p	0.63	0.13	0.12	0.03	0.03	0.74	0.001
	n	137	137	137	137	137	137	137
BNP	r	0.02	0.29	-0.26	-0.03	0.20	-0.06	0.17
	p	0.84	0.00	0.01	0.76	0.03	0.51	0.06
	n	117	117	117	117	117	117	117

Table 3a: Univariate Cox regression analysis of the biomarkers of collagen turnover.

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio Confidence	
MMP-1	1	0.00002	0.00005	0.20	0.65480	1.000	1.000	1.000
MMP-2	1	0.00336	0.00163	4.24	0.03940	1.003	1.000	1.007
MMP-9	1	0.00079	0.00122	0.41	0.52080	1.001	0.998	1.003
TIMP-1	1	0.00055	0.00054	1.07	0.30170	1.001	1.000	1.002
TIMP-2	1	0.01926	0.00605	10.11	0.00150	1.019	1.007	1.032
TIMP-3	1	-0.03070	0.01739	3.12	0.07740	0.970	0.937	1.003
TIMP-4	1	0.00020	0.00011	3.67	0.05530	1.000	1.000	1.000

Table 3b : Univariate prediction power of age, Ejection Fraction, BNP and creatinine using cox regression analysis.

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio Confidence	
Age	1	-0.00283	0.01121	0.06	0.801	0.997	0.976	1.019
Ejection Fraction	1	-0.04107	0.01529	7.22	0.0072	0.960	0.931	0.989
BNP	1	0.0005654	0.0001592	12.61	0.0004	1.001	1.000	1.001
Creatinine	1	0.24243	0.05276	21.1126	<.0001	1.274	1.149	1.413

Table 3c: Univariate Kaplan-Meier Survival analysis of epidemiological variables.

Variable	n (%)	Total N	EVENT %	Log rank test
Female	49 (33%)	147	37%	0.13
Male	98 (67%)		27%	
White	85 (58%)		29.4%	
Black	54 (37%)	147	40.7%	0.52
Other	8 (5%)		25%	

Table 3d: Univariate Kaplan-Meier Survival analysis of Past medical history variables.

Variable	n (%)	Total N	EVENT %		Log rank test
			Yes	No	
History of smoking	40 (27%)	129	42.5%	25.8%	.03
Ischemic Etiology	49 (33%)	146	36%	32%	0.44
Systolic heart failure	145 (99%)	147	NA	NA	NA
Diabetes	49 (33%)	147	42.8%	19.5%	.08
Hypertension	94 (64%)	147	41.4%	20.8%	0.17
Dyslipidemia	75 (51%)	146	40%	25.3%	0.27
Chronic kidney disease	45 (31%)	146	50%	28.8%	.37
Peripheral arterial disease	4 (2.5%)	146	NA	NA	NA
Sleep apnea	30 (20%)	146	63.3%	25.8%	<.001
Depression	25 (17%)	147	48%	30.3%	0.18

Table 3e: Univariate Kaplan-Meier Survival analysis of Treatment and Management Variables

Variable	n (%)	N	EVENT %		Log rank test
			Yes	No	
Beta-blocker	138 (94%)	147	31.9%	55.6%	0.58
ACE inhibitor or angiotensin receptor blocker	123 (84%)	147	31.7%	41.7%	0.69
Statins	78(53%)	147	29.5%	37.7%	0.72
Implantable cardioverter defibrillator and/or biventricular pacemaker	107 (73%)	146	33.7%	31.8%	0.82
Coronary artery bypass graft	28 (19%)	146	39.3%	32.2%	0.58

Table 4a: Linearity of events over TIMP-2 Levels.

TIMP -2 LEVELS pg/ml	1st quartile <108.7	2nd quartile 121.15 – 108.7	3rd quartile 133.82-121.5	4th quartile >133.82
Median levels Pg/ml	102.74	115.43	127.59	147.88
Event rate	18.9%	16.67%	38.16%	59.5%
N	7/37	6/36	14/37	22/37

Adjustment for Multiple Comparisons – Log rank Test				
Strata Comparison		**Chi-Square**	**p-Values**	
TIMP-2	**TIMP-2**		**Raw**	**Sidak**
1	**2**	0.012	0.912	1.000
1	**3**	0.605	0.437	0.968
1	**4**	12.061	0.001	0.003
2	**3**	0.753	0.386	0.946
2	**4**	12.468	0.000	0.003
3	**4**	6.071	0.014	0.080

Table 4b: Comparing Other Outcomes in TIMP-2 groups divided based on linearity.

Timp-2 Groups (> < 75 percentile)	**6-minute walk test (meters) Mean (95%CI)**	**Ejection Fraction Mean (95%CI)**	**KCCQ Summary Score Mean (95%CI)**	**KCCQ Physical limitation Score Mean (95%CI)**
< 133.8 pg/ml (n=110)	382.1 (363 - 401.2) (n=103)	26.3 (24.3 - 28.3) (n=110)	67.5 (62.8 - 72.2) (n=105)	71.6 (67.1 - 75.99) (n=105)
>= 133.8 pg/ml (n=37)	335.2 (302.3 - 368.1) (n=34)	19.3 (15.9 - 22.6) (n=36)	62.2 (55.6 - 68.9) (n=33)	61.6 (54.9 - 68.3) (n=33)
p-value	.016	<.001	NS	.024

Table 5a: Multivariate Cox Regression Analysis

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Age	1	-0.003	0.02	0.03	0.86	1.00	0.96	1.03
Race (White Vs Black)	1	-0.445	0.42	1.15	0.28	0.64	0.28	1.45
Race (White Vs Other)	1	-1.413	0.85	2.78	0.10	0.24	0.05	1.28
Gender	1	-1.259	0.52	5.96	0.01	0.28	0.10	0.78
Smoking History	1	0.911	0.39	5.40	0.02	2.49	1.15	5.36
Depression	1	0.455	0.45	1.01	0.32	1.58	0.65	3.84
Sleep Apnea	1	1.556	0.47	10.77	0.0001	4.74	1.87	12.01
Hypertension	1	0.321	0.45	0.52	0.47	1.38	0.58	3.31
DM	1	0.145	0.41	0.13	0.72	1.16	0.52	2.58
Timp-2 > 133.82	1	1.788	0.51	12.49	0.001	5.98	2.22	16.12
Creatinine	1	0.326	0.07	19.93	<.0001	1.39	1.20	1.60
Sleep Apnea * Timp2 > 133.82	1	-2.254	0.84	7.16	0.01	0.11	0.02	0.55
Ejection Fraction	1	-0.028	0.02	1.74	0.19	0.97	0.93	1.01

Table 5b: The final best fit parsimonious model

Analysis of Maximum Likelihood Estimates								
Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Age	1	0.011	0.013	0.754	0.385	1.011	0.986	1.037
Sleep Apnea	1	1.519	0.411	13.687	0.001	4.568	2.043	10.216
Timp2 > 133.82	1	1.495	0.381	15.379	<.0001	4.460	2.113	9.416
Creatinine	1	0.293	0.060	24.158	<.0001	1.341	1.193	1.508
Sleep Apnea * Timp2 > 133.82	1	-1.481	0.650	5.196	0.023	0.227	0.064	0.813

Figure 1a shows the Kaplan-Meier survival curve of the two groups divide by a cutpoint of TIMP-2 > 133.82 pg/ml. Log Rank test $p < .001$.

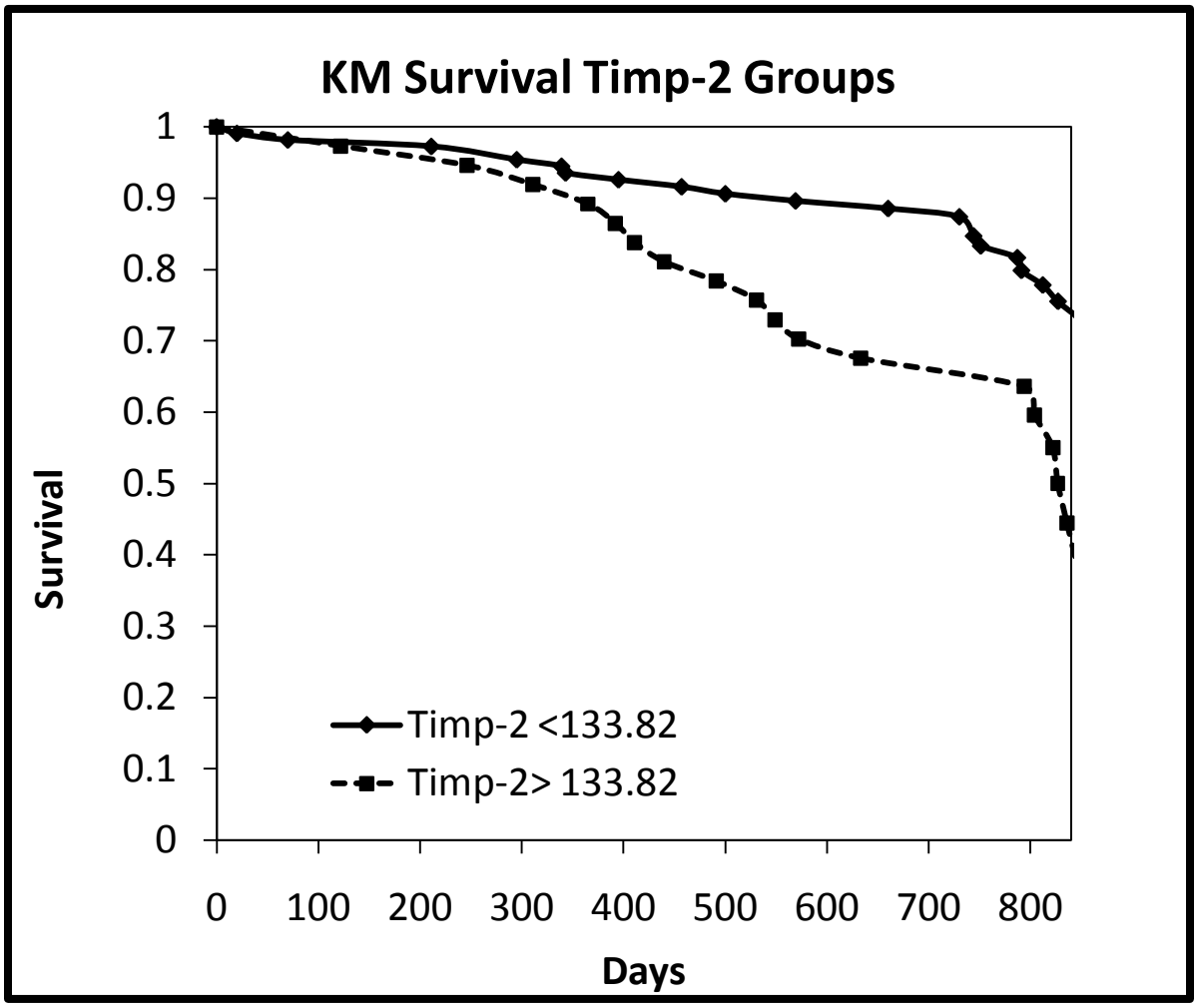
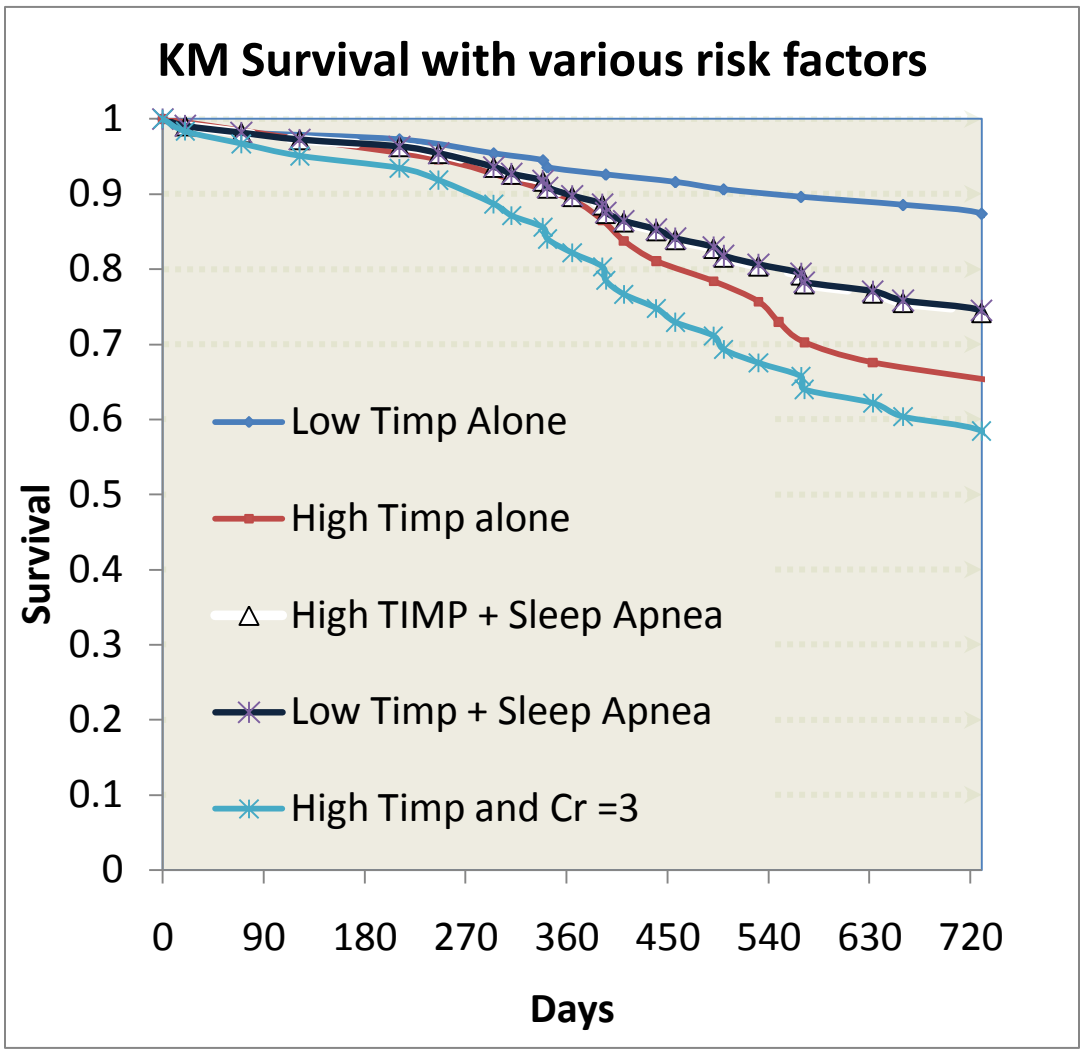


Figure 1b shows the example KM survival curves plotted for a 50 year old individual (with model covariates) to display the outcomes with various risk factors.



References:

1. Hunt, S.A., et al., *ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society.* Circulation, 2005. **112**(12): p. e154-235.
2. Lloyd-Jones, D., et al., *Heart disease and stroke statistics--2010 update: a report from the American Heart Association.* Circulation, 2010. **121**(7): p. e46-e215.
3. He, J., et al., *Risk factors for congestive heart failure in US men and women: NHANES I epidemiologic follow-up study.* Arch Intern Med, 2001. **161**(7): p. 996-1002.
4. Spinale, F.G., *Myocardial matrix remodeling and the matrix metalloproteinases: influence on cardiac form and function.* Physiol Rev, 2007. **87**(4): p. 1285-342.
5. Visse, R. and H. Nagase, *Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry.* Circ Res, 2003. **92**(8): p. 827-39.
6. Spinale, F.G., *Matrix metalloproteinases: regulation and dysregulation in the failing heart.* Circ Res, 2002. **90**(5): p. 520-30.
7. Coker, M.L., et al., *Myocardial matrix metalloproteinase activity and abundance with congestive heart failure.* Am J Physiol, 1998. **274**(5 Pt 2): p. H1516-23.
8. Thomas, C.V., et al., *Increased matrix metalloproteinase activity and selective upregulation in LV myocardium from patients with end-stage dilated cardiomyopathy.* Circulation, 1998. **97**(17): p. 1708-15.
9. Li, Y.Y., et al., *Differential expression of tissue inhibitors of metalloproteinases in the failing human heart.* Circulation, 1998. **98**(17): p. 1728-34.
10. Lopez, B., et al., *Alterations in the pattern of collagen deposition may contribute to the deterioration of systolic function in hypertensive patients with heart failure.* J Am Coll Cardiol, 2006. **48**(1): p. 89-96.
11. Rouet-Benzineb, P., et al., *Altered balance between matrix gelatinases (MMP-2 and MMP-9) and their tissue inhibitors in human dilated cardiomyopathy: potential role of MMP-9 in myosin-heavy chain degradation.* Eur J Heart Fail, 1999. **1**(4): p. 337-52.
12. Jordan, A., et al., *Matrix metalloproteinase-1 and its inhibitor, TIMP-1, in systolic heart failure: relation to functional data and prognosis.* J Intern Med, 2007. **262**(3): p. 385-92.
13. George, J., et al., *Circulating matrix metalloproteinase-2 but not matrix metalloproteinase-3, matrix metalloproteinase-9, or tissue inhibitor of metalloproteinase-1 predicts outcome in patients with congestive heart failure.* Am Heart J, 2005. **150**(3): p. 484-7.
14. Frantz, S., et al., *Tissue inhibitor of metalloproteinases levels in patients with chronic heart failure: an independent predictor of mortality.* Eur J Heart Fail, 2008. **10**(4): p. 388-95.
15. Radauceanu, A., et al., *Extracellular matrix turnover and inflammatory markers independently predict functional status and outcome in chronic heart failure.* J Card Fail, 2008. **14**(6): p. 467-74.
16. Banfi, C., et al., *Neurohormonal activation is associated with increased levels of plasma matrix metalloproteinase-2 in human heart failure.* Eur Heart J, 2005. **26**(5): p. 481-8.
17. Yamazaki, T., et al., *Circulating matrix metalloproteinase-2 is elevated in patients with congestive heart failure.* Eur J Heart Fail, 2004. **6**(1): p. 41-5.

18. Sharma, R. and S.D. Anker, *The 6-minute walk test and prognosis in chronic heart failure--the available evidence.* Eur Heart J, 2001. **22**(6): p. 445-8.
19. Green, C.P., et al., *Development and evaluation of the Kansas City Cardiomyopathy Questionnaire: a new health status measure for heart failure.* J Am Coll Cardiol, 2000. **35**(5): p. 1245-55.
20. Ortega, T., et al., *The utility of a specific measure for heart transplant patients: reliability and validity of the Kansas City Cardiomyopathy Questionnaire.* Transplantation, 2008. **86**(6): p. 804-10.
21. Kosiborod, M., et al., *Identifying heart failure patients at high risk for near-term cardiovascular events with serial health status assessments.* Circulation, 2007. **115**(15): p. 1975-81.
22. Hudson, M.P., et al., *Effects of selective matrix metalloproteinase inhibitor (PG-116800) to prevent ventricular remodeling after myocardial infarction: results of the PREMIER (Prevention of Myocardial Infarction Early Remodeling) trial.* J Am Coll Cardiol, 2006. **48**(1): p. 15-20.
23. Ancoli-Israel, S., et al., *The relationship between congestive heart failure, sleep apnea, and mortality in older men.* Chest, 2003. **124**(4): p. 1400-5.
24. Brew, K. and H. Nagase, *The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity.* Biochim Biophys Acta, 2010. **1803**(1): p. 55-71.
25. Biolo, A., et al., *Episodes of acute heart failure syndrome are associated with increased levels of troponin and extracellular matrix markers.* Circ Heart Fail, 2010. **3**(1): p. 44-50.
26. Brower, G.L., S.P. Levick, and J.S. Janicki, *Inhibition of matrix metalloproteinase activity by ACE inhibitors prevents left ventricular remodeling in a rat model of heart failure.* Am J Physiol Heart Circ Physiol, 2007. **292**(6): p. H3057-64.
27. Li, H., et al., *MMP/TIMP expression in spontaneously hypertensive heart failure rats: the effect of ACE- and MMP-inhibition.* Cardiovasc Res, 2000. **46**(2): p. 298-306.
28. Howard, P.A., et al., *Drug therapy recommendations from the 2005 ACC/AHA guidelines for treatment of chronic heart failure.* Ann Pharmacother, 2006. **40**(9): p. 1607-17.