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Novel and Conventional Biomarkers for Prediction of Myocardial Infarction and Cardiovascular Death

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Abstract

Novel and Conventional Biomarkers for Prediction of Myocardial Infarction and Cardiovascular Death
By Gabrielle Breen

Background: Cardiovascular diseases (CVD) are the leading causes of morbidity and mortality in the United States. Types of CVD include coronary heart disease (CHD), stroke, hypertensive heart disease, inflammatory heart disease, rheumatic heart disease, and other cardiovascular diseases. In literature, scientists have used biomarkers to estimate risk of CVD and risk of CHD, including MI or cardiovascular death. Results from these studies are varied with regards to which biomarkers are associated with an increased risk of CHD. Studies first create a biomarker score from the best biomarkers.
Methods: This analysis is based on 202 enrolled patients who have either a clinical history of angina symptoms, a positive stress test, or have in-stent restenosis (ISR). Patients are enrolled if they have an angiographic ≥ 70 percent, coronary artery or graft stenosis that will be treated with coronary angioplasty and/or stenting procedure. Prior to the interventional procedure, baseline clinical data, including participant's cardiovascular symptom status will be recorded. At the beginning of the angioplasty procedure a blood sample was taken. Blood sampling is repeated in all participants 24 hours post-procedure, one-week post-procedure, and four-weeks post-procedure. Then, the association between the biomarkers and the risk of cardiovascular mortality and myocardial infraction using multivariable Cox Proportional-Hazards Regression models. Then, clinical and multimarker score models with "best-fit" clinical models were compared, based on models containing the clinical risk factors and use of CHD therapies.
Results: During a mean follow up of 6.3 years, 49 (24%) participants had a MI or died from cardiovascular diseases. Using backwards elimination with retention p-value=0.20, these remained in the model: triglycerides, serum creatinine, and biomarkers, SDF-1, TNF, and CRP. For the endpoint of a MI or cardiovascular death the multimarker score with biomarkers SDF-1, TNF, and CRP is: $H = (3.668 * \text{SDF} - 1) + (0.345 * \text{TNF}) + (0.314 * \text{CRP})$.
Conclusions: Concentrations of SDF-1, TNF, and CRP help to predict the future risk of MI or CV death still in the context of robust clinical risk models. Addition of these biomarkers improves discrimination. These biomarkers can assist with prognostic value for identifying death or MI after undergoing angioplasty.

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Table of Contents

List of Tables

List of Figures

1. Introduction

Cardiovascular diseases (CVD) are the leading causes of morbidity and mortality in the United States (Vasan, 2006).

Because of the burden CVD in the US, primary and secondary prevention is extremely important in public health. CVD is a class of diseases that involve the heart and blood vessels. Types of CVD include coronary heart disease (CHD), stroke, hypertensive heart disease, inflammatory heart disease, rheumatic heart disease, and other cardiovascular diseases (Mendis, 2011).

Annually, in the United States, approximately one in four deaths are attributed to CHD (Heart Disease Facts, 2012). Those without CHD have an average lifespan ten years greater than those who suffer from CHD (What Are Coronary Heart Disease Risk Factors?, 2011).

CHD is a disease in which plaque, a waxy substance, builds up inside the coronary arteries. These arteries supply oxygen-rich blood to the heart. Over many years, the plaque can build up, which is a condition called atherosclerosis (What Is Coronary Heart Disease?, 2013). Atherosclerosis, over time, can harden or rupture which reduces the oxygen flow to the heart. If the plaque ruptures, a blood clot can form on the surface restricting or eliminating blood flow through the coronary artery. If this happens, a heart attack, or myocardial infraction (MI), can occur (What Is Coronary Heart Disease?, 2013).

One in five men who had a first MI died within one year, a mortality rate 14 times greater than that of those free of CHD (Heart Disease Facts, 2012). Men who have a first MI are four times that of the general population to have a second MI (Heart Disease

Facts, 2012). After a first MI, within five years is 25 percent mortality; three times that of the general population (Heart Disease Facts, 2012). In the Framingham Study, a second MI occurred in 13 percent of the men and 40 percent of the women within five years of the first MI (Heart Disease Facts, 2012).

1.1. Clinical Risk Factors of CHD

Epidemiological research has identified cigarette smoking, diabetes, hyperlipidemia, and hypertension as independent risks for CHD (Kannel, 1979). Additional clinical risk factors often identified for CHD include body mass index, lack of physical activity, diet, stress, age, gender, and family history (Kannel, 1979).

While these are considered conventional risk factors, it is estimated that less than 50 percent of patients with CHD lack these risk factors (Kannel, 1979). In patients with CHD, conventional risk factors were present at a higher prevalence than commonly believed with only 15 percent to 20 percent of patients lacking any of the conventional risk factors (Kannel, 1979).

1.2. Inflammation and CVD

Inflammation is an important causative factor across the spectrum of acute and chronic phases of vascular disease. Chronic endothelial injury, an underlying cause of CHD, induces the expression of intercellular adhesion molecules and the release of chemoattractant compounds that mediate the recruitment, attachment, and migration of leukocytes into the arterial wall. Infiltrating inflammatory cells enhance oxidation and uptake of low-density lipoproteins and produce cytokines, mitogens, and reactive oxygen

species, stimulating smooth muscle cell migration and proliferation and contributing to ongoing endothelial injury (Ross, 1993).

1.3. Biomarkers and CVD Risk

Biological markers (biomarkers) were introduced as a Medical Subject Heading term in 1989 as "'measurable and quantifiable biological parameters (e.g. specific enzyme concentration, specific gene phenotype distribution in a population, presence of biological substances) which serve as indices for health- and physiology-related assessments, such as disease risk, psychiatric disorders, environmental exposure and its effects, disease diagnosis, metabolic processes, substance abuse, pregnancy, cell line development, epidemiological studies, etc.'" (Vasan, 2006). Biomarkers are used to assist the clinician to optimally manage the patient.

In literature, scientists have used biomarkers to estimate risk of CVD and risk of CHD, including MI or cardiovascular death. Results from these studies are varied with regards to which biomarkers are associated with an increased risk of CHD. Studies first create a biomarker score from the best biomarkers (Blankenberg, 2010) (Wang, 2012). Then depending on the clinical question of interest, scientists model risk factors plus the biomarker score, including Cox Proportional Hazard and Logistic Regression models depending on the question of interest (Blankenberg, 2010) (Wang, 2012). In this paper, a Cox Proportional Hazard Model is used to determine association between risk factors and cardiovascular death or myocardial infarction because of the interest in years to event. Using data from the Framingham Heart Study, biomarkers add prognostic value to standard risk factors for predicting death, overall CVD events, and heart failure whereas

in post-menopausal women, moderate improvement in CHD risk prediction was found when an 18-panel biomarker panel was added to predictive models using traditional risk factors (Wang, 2012) (Kim, 2010).

Serum markers of inflammation have been found to be prognostic for the prevalence of atherosclerosis, clinical manifestations of coronary artery disease (CAD), and increased risk for complications after acute ischemic syndromes or coronary interventional procedures. Prospective and cross-sectional studies have documented associations between levels of CRP in apparently healthy individuals and the occurrence of myocardial infarction, stroke, or cardiovascular mortality (Ridker, 1997) (Ridker, 2003). Among patients with acute ischemic syndromes, elevated circulating concentrations of CRP, IL-1 receptor antagonist, or IL-6 are predictive of recurrent ischemia, myocardial infraction, and long-term mortality (Libb, 2002). This analysis will include these biomarkers as well as others to determine association with myocardial infarction or cardiovascular death.

1.4. Study Purpose

MI's are often caused by atherosclerotic coronary artery disease, a hardening of the arteries. While patients may present with atherosclerotic coronary artery disease the prediction of MI or death among individuals without all clinical risk factors is an important challenge. Identifying which biomarkers are associated with MI or death in these individuals already presenting with atherosclerotic coronary artery disease is paramount.

Patients presenting with atherosclerotic coronary artery disease are at high risk for

an MI; having the ability to predict what characteristics are associated with an MI or death, after presenting with atherosclerotic coronary artery disease can greatly affect the lives of those affected by CHD. Clinical risk factors, while associated with CHD, may not be present in patients presenting with atherosclerotic coronary artery, so identifying biomarkers can help identify associations between patients and the outcome of MI or cardiovascular death. Using clinical risk factors and biomarker risk score, a Cox Proportional Hazards model will be estimated to determine the association between risk factors and biomarker score and time to cardiovascular death or MI.

1.5. Treatment and Interventions

Abciximab, a potent platelet glycoprotein IIb/IIIa receptor blocker, markedly decreases the risk of ischemic complications from coronary interventions such as angioplasty and stenting when used in the peri-procedural settings. Trials have shown reduced risk of death, myocardial infraction, or emergency repeat revascularization 30 days after coronary intervention, with a mortality benefit extending out to a year or more (Topol, 1999).

2. Methods

2.1. Study Sample

After approval by Emory University's Institutional Review Board, patients were invited to enroll into the *Anti-inflammatory effects of abciximab (Reopro)* study if they presented to either Emory University Hospital or the Atlanta Veterans Administration Hospital with atherosclerotic coronary artery disease that are undergoing angioplasty, aiming to enroll 125 with diabetes and 125 without diabetes.

This analysis is based on 202 enrolled patients who have either a clinical history of angina symptoms, a positive stress test, or have in-stent restenosis (ISR). Patients are enrolled if they have an angiographic ≥ 70 percent, coronary artery or graft stenosis that will be treated with coronary angioplasty and/or stenting procedure. Participants are ≥ 18 years old. Prior to the procedure, participants were on a stable HMG-CoA-reductase inhibitor (statin) dose for at least 4 weeks before the angioplasty/stent procedure.

Participants underwent a standardized screening of review of history, physical, and lab studies for eligibility. Additionally, participants had an evaluation assessing the diagnostic coronary angiogram and ventriculogram for eligibility. Participants also had their blood drawn prior to the interventional procedure and the procedure was repeated twenty-four hours post-procedure, one week post-procedure, and four weeks post-procedure.

Participants undergoing coronary intervention were either assigned to receive intravenous abciximab and heparin, heparin only, or bivalirudin based on the interventional cardiologist's preference. The participant was not informed of his

treatment assignment. Participants in the control group only underwent cardiac catheterization and did not receive abciximab, bivalirudin, or heparin.

Following the procedure, participants received their usual customary medical therapy following the interventional procedure. The dose of statins will not be altered for four weeks following the onset of the study.

2.2. Biomarker Measurements

Prior to the interventional procedure, baseline clinical data, including participant's cardiovascular symptom status will be recorded. At the beginning of the angioplasty procedure a blood sample was taken. Blood sampling is repeated in all participants 24 hours post-procedure, one-week post-procedure, and four-weeks post-procedure. The biomarkers measured in this study are listed and defined in **Table 1**.

2.3. Outcomes

Participants were contacted by letter or telephone call to assess health beyond the four-week follow-up. The primary endpoint in this study was the number of participants necessary to evaluate for a reduction in inflammatory markers with treatment of abciximab.

2.4. Statistical Analyses

Before inferential analyses, biomarker values were natural log transformed due to highly skewed distributions. The partial correlations among biomarkers accounting for gender and age were estimated to determine the association among the biomarkers. Then,

the association between the biomarkers and the risk of cardiovascular mortality and myocardial infraction using multivariable Cox Proportional-Hazards Regression models were examined rather than logistic regression because of the interest in time to event. The proportionality assumption was assessed by testing the interaction of the biomarkers with the follow-up time.

The Cox Proportional-Hazards Regression Model is:

$h(t|X) = h(t)\exp(X_1\beta_1 + \cdots + X_p\beta_p)$, where the predictors $X_1, \ldots, X_p$ are assumed to behave additively on $\log h(t|X)$, and the effect of the predictors $X_1, \ldots, X_p$ are the same for at all times t. The parameters $\beta_1, \ldots, \beta_p$ of the model are estimated by maximizing the partial likelihood, which is given by

$$L(\beta) = \prod_{Y_i\ uncensored} \frac{\exp(X_i\beta)}{\sum_{Y_j \geq Y_i} \exp(X_j\beta)}.$$

The log partial likelihood is given by

$$l(\beta) = logL(\beta) = \sum_{Y_i\ uncensored}\{X_i\beta - \log\left[\sum_{Y_j \geq Y_i} exp(X_j\beta)\right]\}.$$

Then, the hazards ratios and confidence limits can be estimated using maximum likelihood estimators.

In these analyses the cases with no missing data (N=88) were used. Biomarkers were assessed individually in models containing the following standard cardiovascular risk factors: age, gender, race, diabetes, cigarette smoking, hypertension, hypercholesterolemia, use of statin, cholesterol, previous MI, left ventricular ejection fraction, serum creatinine, troponin I, and triglycerides. Also the therapy of interest in this study, the use of abciximab, was included in the model. The biomarkers il-1ra, il-1β,

IL-6, VEGF, PlGF, FORT, GCSF, FGF, HGF, and GM-CSF were excluded because of the number of missing observations.

Sex-pooled analyses were performed after confirming that multiplicative interaction terms for biomarker and gender were statistically non-significant. Analyses were performed incorporating all biomarkers together. The identification of the most strongly associated biomarkers for death or MI was confirmed using backwards elimination model using a retention p-value of 0.20.

The joint utility of the biomarkers was evaluated by constructing a "multimarker" risk score. This score was defined as:

$$H = (\beta_1\ x\ \text{biomarker A}) + \cdots + (\beta_q\ x\ \text{biomarker q})$$

with $\beta_1, \ldots, \beta_q$ denoting proportional-hazards regression coefficients for biomarkers $A_1,\ldots,A_q$ respectively, from a multivariable model for the composite outcome of death or MI (Wang, 2012). Participants were categorized according to specific quartiles of this multimarker score.

Then, clinical and multimarker score models with "best-fit" clinical models were compared, based on models containing the clinical risk factors and use of CHD therapies. The performance was assessed using current methods utilizing a Rocplus Macro (Pencina, 2008) (Pencina and Bergstralh, 2008). First, the model discrimination was evaluated using c-statistics for models including clinical predictors listed with and without the biomarkers. C-statistics are a weighted average of sensitivity over all possible end-points. Models are considered reasonable when the c-statistic exceeds 0.70 and strong when the c-statistic exceeds 0.80 (Hosmer, 2000).

Then, the integrated discrimination improvement (IDI), a measure of a model's ability to improve average sensitivity without reducing average specificity, was calculated (Pencina, 2008) (Pencina and Bergstralh, 2008). IDI uses probability differences rather than event and non-event categories. IDI is the "difference between improvement in average sensitivity and any potential increase in average 'one minus specificity'" (Pencina, 2008). IDI is defined as:

$$\mathrm{IDI} = (\mathrm{IS}_{\mathrm{new}} - \mathrm{IS}_{\mathrm{old}}) - (\mathrm{IP}_{\mathrm{new}} - \mathrm{IP}_{\mathrm{old}}),$$

where IS is the integral of sensitivity over cut-off values in the range (0,1) and IP is the integral of 'one minus specificity' for the new and old models. IDI is the probability difference in discrimination slopes i.e. the mean difference in probability between death and MI and not death and not MI.

Then, the ability of biomarkers to reclassify risk was evaluated, by examining the proportion of individuals reclassified correctly using the net reclassification improvement (NRI). Unlike IDI, NRI uses the proportion of participants with the endpoint death or MI and those who do not die or have a MI. Participants in the group of death or MI are assigned 1 for upward movement, -1 for downward movement, and 0 for no movement. The opposite is assigned to participants in the non-event group (Pencina, 2008).. Each group's reclassification number assignments are summed and divided by the number of participants in each group. NRI is the difference in these quantities. NRI can be interpreted as the net gain or net loss in the reclassification proportion. NRI is calculated by:

$$\mathrm{NRI} = [\mathrm{P}(\mathrm{up}|\mathrm{D} = 1) - \mathrm{P}(\mathrm{down}|\mathrm{D} = 1)] - [\mathrm{P}(\mathrm{up}|\mathrm{D} = 0) - \mathrm{P}(\mathrm{down}|\mathrm{D} = 0)]$$

(Pencina, 2008).

The NRI is calculated using 0%, 1%, and 2%, to compare thresholds for minimum change in predicted risk required to indicate a change in classification.

All statistical procedures were carried out with the SAS statistical software package (release 9.3, 2012, SAS Institute Inc., Cary, NC). The Rocplus macro was used for the calculation of the area under curve and the net reclassification improvement of models predicting outcome events (Pencina and Bergstralh, 2008).

3. Results

3.1. Participant Characteristics

Characteristics and biomarkers measured at baseline are summarized in **Table 2**. Of the 202 patients in the study population, 82.67% (n=167) were male. The ages ranged from 32 to 84 (median age = 61 years old). Of the 82% (n=166) who were not African American, 2 were Asian, 153 were Caucasian, 2 were Hispanic, 4 were other, and 5 were missing values for race. Only 27.3% reported as current smokers and 46.9% reported as former smokers. The mean BMI is 29. 7, with the lowest 25% of the participants with BMI $\leq$ 26.26, the middle 50% in the range of (26.25, 29.27), the third quartile in the range (29.27, 32.85), and the fourth quartile in the range (32.85, 45.04). Underweight is defined as BMI < 18.5, normal weight is in the range 18.5-24.9, overweight is defined as BMI in the range 25-29.9, and obese is a BMI $\geq$ 30. Forty-four percent (n=89) had diabetes mellitus. The mean high-density lipoprotein is 41.9 and the mean high-density lipoprotein is 95.3. Eighty-one percent of participants had hypertension and 88 percent suffered from hypercholesterolemia. Approximately 80 percent were treated with statin, an inhibitor prescribed to lower cholesterol levels. Forty-seven percent were treated with abciximab, a drug used to prevent the clumping of blood-clotting cells in the blood.

3.2. Baseline Biomarkers and Clinical Characteristics

Most of the biomarkers demonstrated mostly modest pairwise correlation adjusting for age and gender, with correlation coefficients ranging from -0.14 to 0.34 summarized in **Table 3**. Stronger correlations were evident for TNF and mcp1 (Spearman ρ=0.33, p-value < 0.0001), sCD40L and SDF-1 (Spearman r=0.34, p-value < 0.0001),

sCD40L and CRP (Spearman r=0.24, p-value 0.02), and MMP-9 and CRP (Spearman r=0.29, p-value 0.01).

3.3. Prediction of MI and death with new biomarkers

During a mean follow up of 6.3 years, 49 (24%) participants had a MI or died from cardiovascular diseases. Associations of sCD40L, mcp1, IL-10, TNF, MMP-3, MMP-9, SDF-1, and CRP with CV death or MI are shown in **Table 4**. Then, models were evaluated adjusting for age, gender, race, diabetes, cigarette smoking, hypertension, hypercholesterolemia, use of statin, cholesterol, previous MI, left ventricular ejection fraction, serum creatinine, troponin I, and triglycerides. The therapy of interest in this study, the use of abciximab, was also included. SDF-1 provides strong evidence of association with a MI or death (p-value < 0.05) while mcp1 and IL-10 demonstrate moderate evidence of association with a MI or death (0.05 < p-value < 0.07). The Survival Curves are in **Figures 1-9**.

Then using backwards elimination with retention p-value=0.20, these remained in the model: triglycerides, serum creatinine, and biomarkers, SDF-1, TNF, and CRP.

3.4. Multimarker Score

For the endpoint of a MI or cardiovascular death, a multimarker score, with biomarkers SDF-1, TNF, and CRP was created. The score is: $H = (3.668 * \text{SDF} - 1) + (0.345 * \text{TNF}) + (0.314 * \text{CRP})$.

Table 5 shows the incremental predictive value of the multimarker scores with the clinical risk factors in the final model. Participants in the fourth quartile had

approximately 1-fold risk of MI or CV death, in the second quartile 7-fold risk for MI or CV death, and in the third quartile 2.5-fold risk of MI or CV death, compared with participants in the lowest quartile.

3.5. IDI and NRI

The addition of the multimarker score in the Cox Proportional Hazards Model did not lead to an improvement in discrimination by statistically insignificant increases in the c-statistic (p-value = 0.11) and integrated discrimination improvement (p-value=<0.001) seen in **Table 5**.

Those who did not experience an endpoint of an event of CV death or a MI, NRI values were 0.41 (p-value < 0.001). The NRI maintained statistical significance when a different minimum threshold for change in predicted risk was used. NRI (>0.01) was 0.31 (p-value=0.0032) whereas when minimum threshold for change in predicted risk was 0.02, the NRI was marginally statistically significant. NRI (>0.02) was 0.20 (p-value = 0.06).

4. Conclusions, Implications, and Recommendations

4.1. Summary of Findings

While clinical risk factors are used to predict who is most likely to have a MI or die from cardiovascular diseases after undergoing angioplasty, identifying other methods to determine who is at risk is an important task; biomarkers are used in cancer, cardiovascular, and diabetes research although in cardiovascular literature there are differing conclusions regarding what biomarkers are associated with various outcomes. Concentrations of multiple biomarkers provide prognostic information in addition to what clinical risk factors can provide. In addition to the clinical risk factors, in this cohort, the biomarkers SDF-1, TNF, and CRP are in the final model.

4.2. Clinical Significance

Stromal-derived factor-1 (SDF-1) is thought to promote the growth, survival, and development of bone marrow stem cells (Kortesidis, 2005). Tumor Necrosis Factor-Alpha (TNF) is a cytokine that is produced in white blood cells. The primary role of Tumor Necrosis Factor (TNFα) is the regulation of immune cells (Szlosarek, 2003). C-reactive protein (CRP) is produced by the liver and the level of CRP increases when there is inflammation in the body (C-reactive protein test, 2011).

As these three biomarkers are associated with a MI or CV death these three biomarkers also have unique aspects of pathophysiology. This idea is further supported by the relatively low correlations between the biomarkers (**Table 3**), indicating that each biomarker provides unique information (Gerszten, 2008).

4.3. Study Strengths

This is a first in analyzing biomarkers among patients undergoing angioplasty with the interest of an outcome of myocardial infarction or cardiovascular death. An advantage of modeling Cox Proportional Hazard models is that censoring is not an issue in the analysis. Although this analysis with clinical risk factors and the addition of the multimarker score did not lead to an improvement in discrimination there is additional value in utilizing biomarkers for the prediction of MI or cardiovascular death. While in literature, biomarkers for predicting cardiovascular events in community-based populations have not consistently added information to clinical risk factors (Wang, 2012), this analysis is one of the first to determine how biomarkers add to MI or death prediction after the CHD is present in patients requiring angioplasty.

4.4. Study Limitations

This study aimed to enroll 250 individuals at two hospital locations but ultimately only enrolled 202 participants. Of the 202 with complete data for the variables of interest was N=88 which may bias the results; we also assume that the missing data are missing at random. Future studies can hopefully provide additional observations, particularly for the biomarkers excluded in this analysis. Additionally, this study was interested in the affect of abciximab but was not a double-blinded study and rather abciximab was prescribed by doctor discretion.

4.5. Future Studies

Additionally, a similar analysis can be extended to other clinical endpoints such as stroke, revascularization, or cardiovascular death. Although we see some improvement in prediction of death or MI using the biomarker scores, further analyses should be conducted to explore the association between the changes, and directional changes, in the biomarkers over the four measurements are associated with MI or death. This analysis included CVD risk factors as well as abciximab; other analyses can be done to identify affect of abciximab on the outcome of cardiovascular death or MI. To address the issue of the missing data, future analysis can incorporate multiple imputation methods which estimate the missing values.

4.6. Conclusion

Concentrations of SDF-1, TNF, and CRP help to predict the future risk of MI or CV death still in the context of robust clinical risk models. Addition of these biomarkers improves discrimination. These biomarkers can assist with prognostic value for identifying death or MI after undergoing angioplasty.

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Appendix

Table 1. Biomarkers: A Basic Glossary

sCD40L	Soluble CD40 ligand
il-1ra	Interleukin-1 receptor antagonist
il-1β	Interleukin-1 beta
mcp1	Monocyte chemotactic protein-1
IL-6	Interleukin-6
IL-10	Interleukin-10
TNF	Tumor Necrosis Factor (TNFα)
VEGF	Vascular Endothelial Growth Factor
MMP-3	Matrix metalloproteinase-3
MMP-9	Matrix metallopeptidase-9
SDF-1	Stromal cell-derived factor-1
PlGF	Placenta Growth Factor
CRP	C-Reactive Protein
GCSF	Granulocyte colony-stimulating factor
FGF	Fibroblast growth factors
HGF	Hepatocyte growth factor
FORT	Free Oxygen Radical Test
GM-CSF	Granulocyte-macrophage colony-stimulating factor

Table 2. Baseline Characteristics of the Study Sample

Characteristic	Total Study Sample (n=202)	Sample Excluding Participants with endpoint of MI or CV death (n=153)
Male, %	82.7	81.7
Age	61.3±9.8	60.6±9.4
Body Mass Index, kg/m^2	29.7±5.8	29.7±5.8
Race, %		
Caucasian	77.7	74.3
African American	18.3	20.3
Asian	1.0	1.4
Hispanic	1.0	1.4
Treated with Abcixim, %	47.2	46.0
Stroke, %	4.0	3.3
Diabetes, %	44.5	44.1
Hypertension, %	83.8	82.0
Hypercholesterolemia, %	88.9	88.1
Cigarette Smoking Current, %	27.3	25.9
Treated with Statin, %	77.9	74.8
Serum creatinine, mg/dL	1.0±0.3	1.0±.2
Troponin I	0.1±0.3	0.1±0.2
Glucose	127.3±45.9	125.8±42.8
Cholestoral	173.2±41.9	174.6±41.8
Low-density lipoprotein	95.3±33.3	96.1±33.6
High-density lipoprotein	41.9±10.6	42.3±10.9
Triglycerides	189.6±172.6	181.7±149.6
Left ventricular ejection fraction	52.2±9.2	53.1±9.0
Biomarkers, median (quartiles 1, 3)		

IL-10	0.3 (0.2, 0.5)	0.3 (0.2, 0.6)
TNF	3.0 (1.8, 4.3)	2.8 (1.6, 4.0)
MMP-3	9.5 (7.0, 13.3)	8.9 (6.3, 12.6)
MMP-9	77.5 (41.1, 160.2)	83.3 (40.4, 172.5)
SDF-1	1,619.5 (1,268.4, 2,015.1)	1,497.1 (1,234.1, 1,891.0)
CRP	3.0 (1.1, 5.3)	3.0 (1.1, 4.9)

For continuous variables, values are mean±SD or medians(quartile 1, quartile 3).

Table 3. Age- and Gender- Adjusted Correlations Among Biomarkers

	sCD40L	mcp1	IL-10	TNF	MMP-3	MMP-9	SDF-1	CRP
sCD40L	1.00	...	...	...	...	...	...	...
	...	...	...	...	...	...	...	...
mcp1	0.00	1.00	...	...	...	...	...	...
	0.99	...	...	...	...	...	...	...
IL-10	-0.09	0.10	1.00	...	...	...	...	...
	0.41	0.34	...	...	...	...	...	...
TNF	0.13	*0.33*	0.05	1.00	...	...	...	...
	0.23	*0.00*	0.67	...	...	...	...	...
MMP-3	-0.04	0.10	0.04	-0.06	1.00	...	...	...
	0.70	0.37	0.69	0.60	...	...	...	...
MMP-9	0.14	0.17	-0.11	0.03	-0.03	1.00	...	...
	0.21	0.12	0.31	0.77	0.77	...	...	...
SDF-1	*0.34*	0.12	-0.10	0.09	0.04	0.04	1.00	...
	0.00	0.29	0.35	0.41	0.74	0.68	...	...
CRP	*0.24*	0.09	0.02	0.19	-0.14	*0.29*	0.03	1.00
	0.02	0.39	0.89	0.08	0.20	*0.01*	0.80	...

Values are age- and gender- adjusted Spearman correlation coefficients (N = 91).
All biomarkers are natural log transformed.
Values are correlation coefficients and p-values.

Table 4. Association of New Cardiac Biomarkers With MI or CV Death, Before Model Selection.

Individual Biomarkers*	HR	HRCL		P-value
sCD40L	1.344	0.777	2.325	0.291
mcp1	3.819	0.927	15.735	0.064
IL-10	1.044	0.730	1.493	0.056
TNF	1.564	0.903	2.709	0.110
MMP-3	1.625	0.403	6.558	0.495
MMP-9	1.037	0.585	1.840	0.900
SDF-1	42.601	5.223	357.469	>0.0001
CRP	1.262	0.869	1.832	0.221

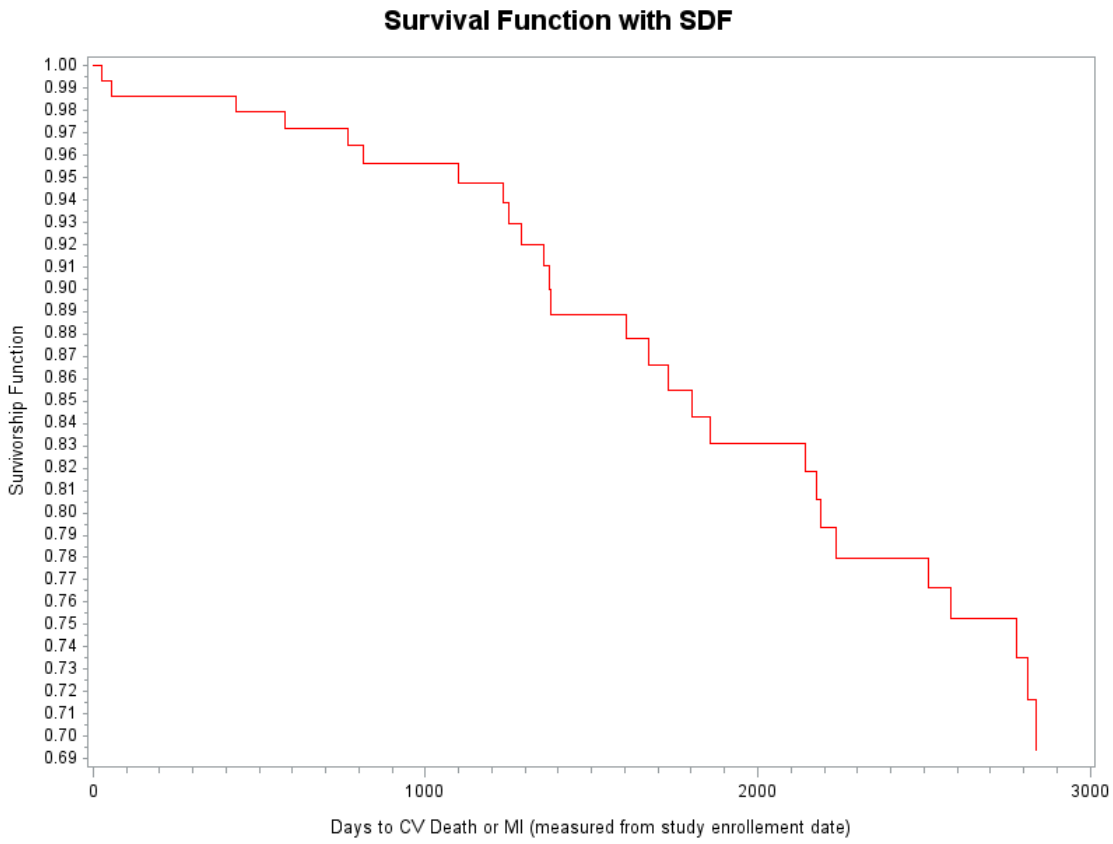
*Biomarkers are transformed on natural log scale

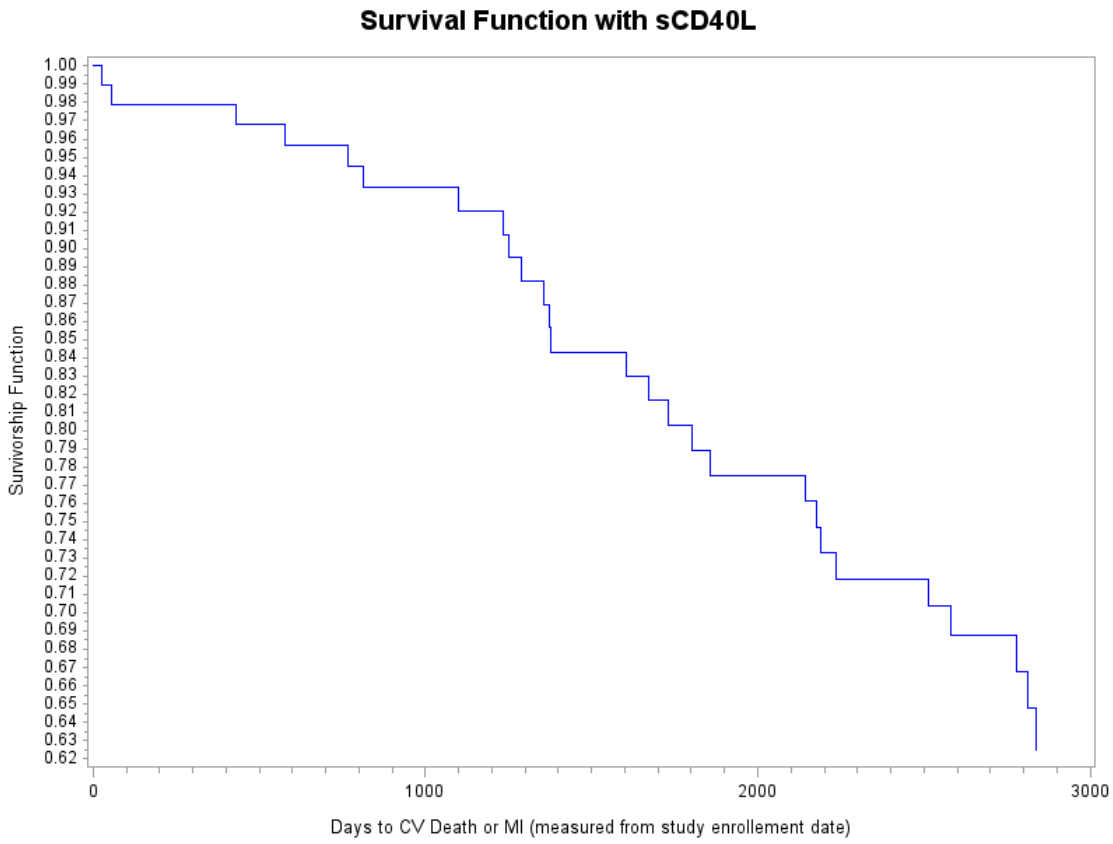
Values are Hazard Ratios (HR) and 95% confidence limits (HRCL), from models adjusting for clinical factors including age, gender, race, use of Abciximab, diabetes, hypertension, hypercholesterolemia, cigarette smoking, use of statin, cholesterol, triglycerides, previous MI, troponin I, serum creatinine, and left ventricular ejection fraction.

Table 5. Multimarker Score and prediction of MI or CV death

	NRI* (>0)
Score, per 1-unit increment	1.4 (0.7, 3.0)
p-value	0.3
By quartile of score	
1st quartile	Referent
2nd quartile	6.9 (0.7, 68.7)
3rd quartile	2.5 (0.2, 27.4)
4th quartile	0.7 (0.0, 12.2)
P for trend	0.02
c-statistics	
Best-fit clinical model	0.75
Best-fit clinical model + multimarker score	0.86
p-value	0.11
IDI	0.18
p-value	<0.001
NRI, versus best-fit clinical model	0.41
p-value	<0.0001

Values for continuous score and quartiles are hazard ratios, with 95% CI, from multivariable models adjusting for triglycerides and serum creatinine. SD. Standard Deviation; IDI: integrated discrimination improvement; NRI: net reclassification improvement.
*NRI(>0) denotes category-free NRI, using a threshold of 0% for the minimum change in predicted risk necessary to change reclassification.


Survival Function with SDF
Survivorship Function
1.00
0.99
0.98
0.97
0.96
0.95
0.94
0.93
0.92
0.91
0.90
0.89
0.88
0.87
0.86
0.85
0.84
0.83
0.82
0.81
0.80
0.79
0.78
0.77
0.76
0.75
0.74
0.73
0.72
0.71
0.70
0.69
0
1000
2000
3000
Days to CV Death or MI (measured from study enrollement date)


Survival Function with sCD40L
Survivorship Function
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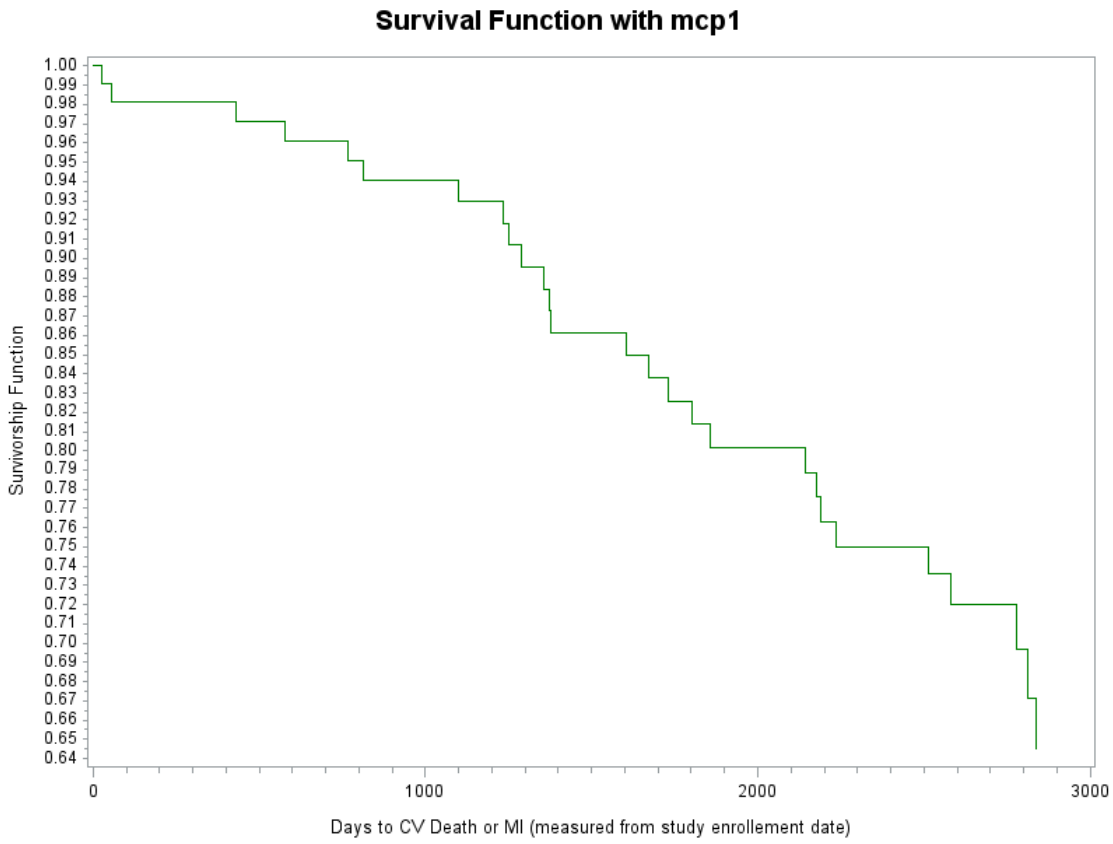
0.99
0.98
0.97
0.96
0.95
0.94
0.93
0.92
0.91
0.90
0.89
0.88
0.87
0.86
0.85
0.84
0.83
0.82
0.81
0.80
0.79
0.78
0.77
0.76
0.75
0.74
0.73
0.72
0.71
0.70
0.69
0.68
0.67
0.66
0.65
0.64
0.63
0.62
0
1000
2000
3000
Days to CV Death or MI (measured from study enrollement date)


Survival Function with mcp1
Survivorship Function
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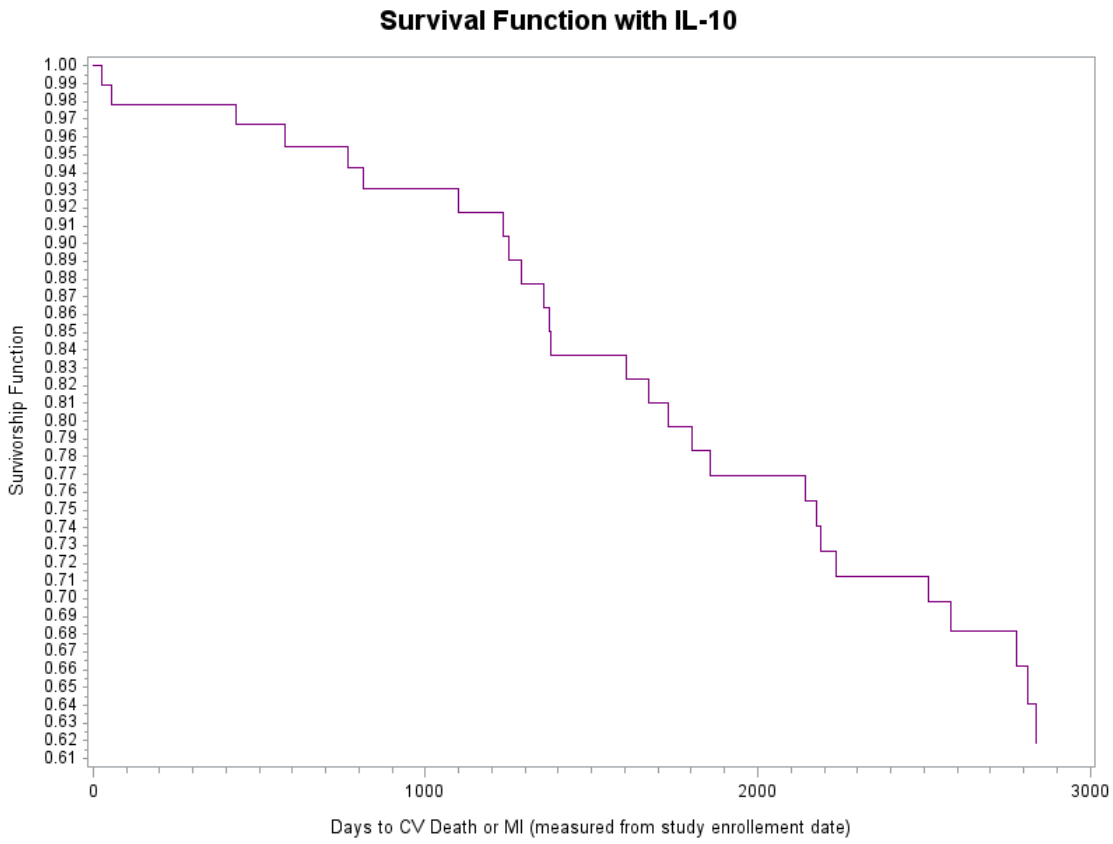
0.99
0.98
0.97
0.96
0.95
0.94
0.93
0.92
0.91
0.90
0.89
0.88
0.87
0.86
0.85
0.84
0.83
0.82
0.81
0.80
0.79
0.78
0.77
0.76
0.75
0.74
0.73
0.72
0.71
0.70
0.69
0.68
0.67
0.66
0.65
0.64
0
1000
2000
3000
Days to CV Death or MI (measured from study enrollement date)


Survival Function with IL-10
Survivorship Function
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0.98
0.97
0.96
0.95
0.94
0.93
0.92
0.91
0.90
0.89
0.88
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0.85
0.84
0.83
0.82
0.81
0.80
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0.72
0.71
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0.69
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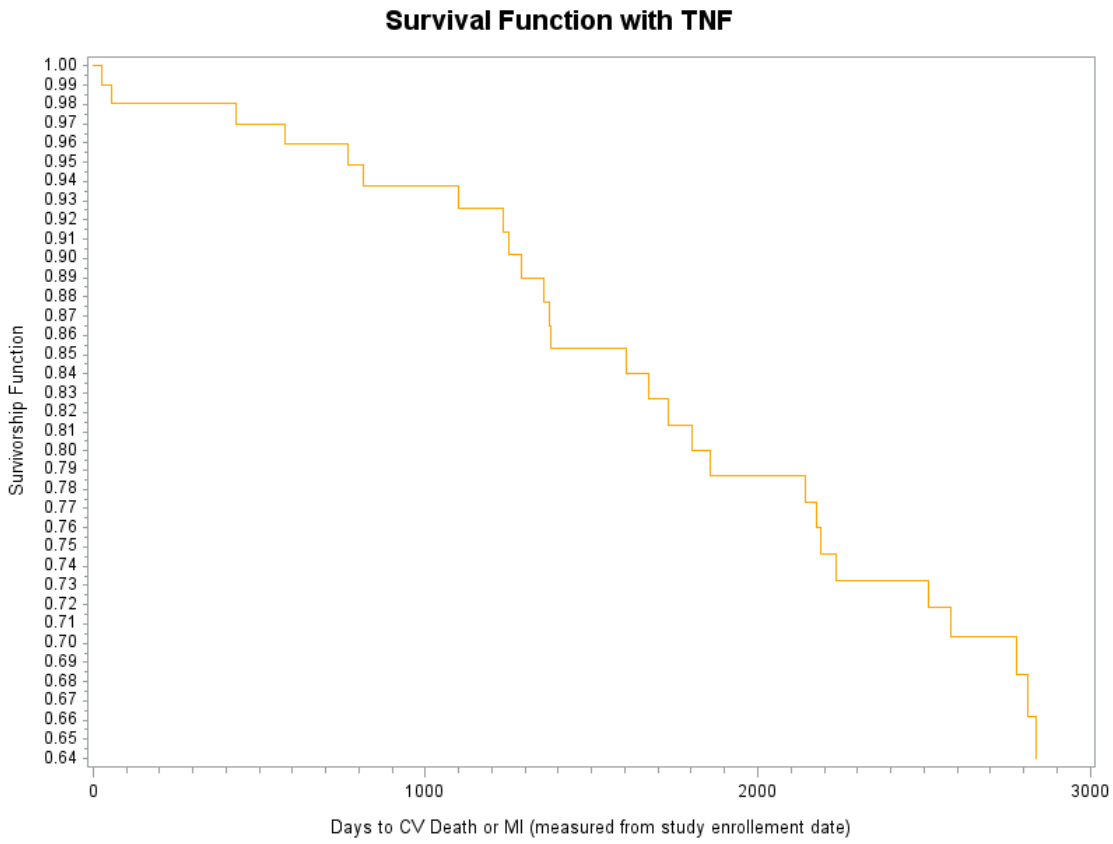
0.64
0.63
0.62
0.61
0
1000
2000
3000
Days to CV Death or MI (measured from study enrollement date)


Survival Function with TNF
Survivorship Function
1.00
0.99
0.98
0.97
0.96
0.95
0.94
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0.89
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0.64
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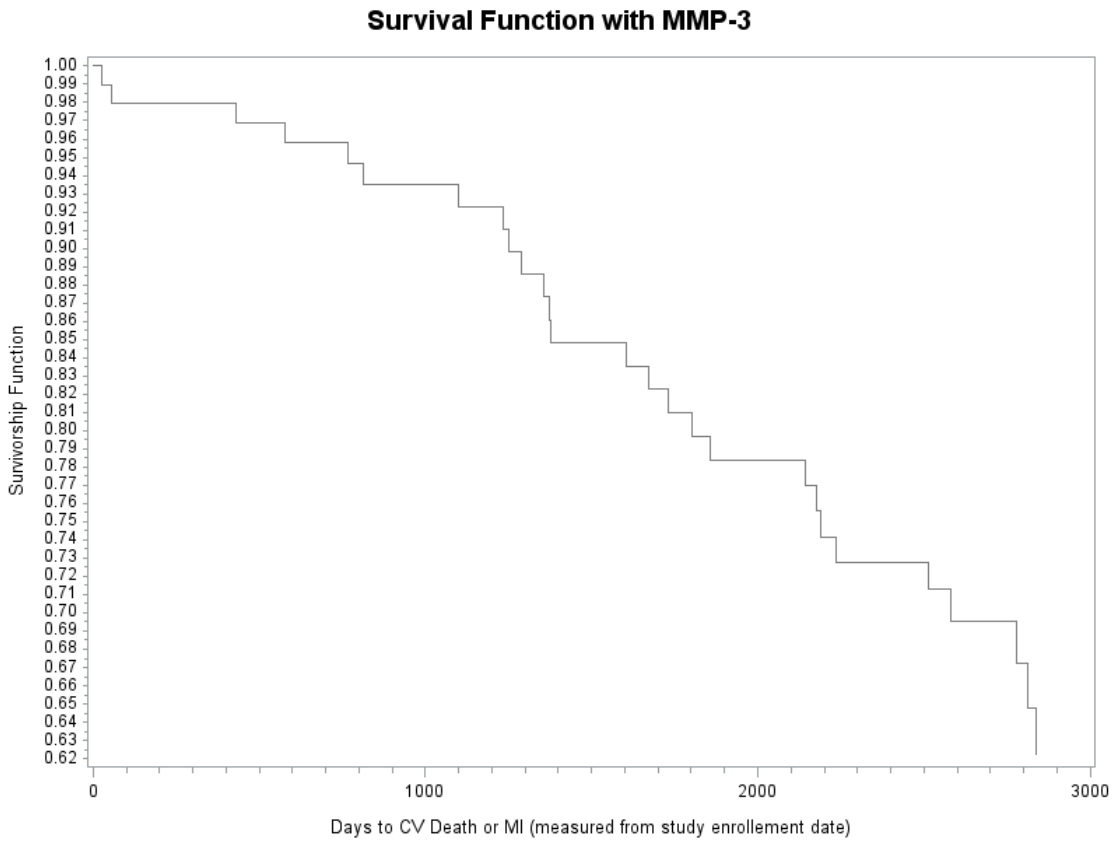
1000
2000
3000
Days to CV Death or MI (measured from study enrollement date)


Survival Function with MMP-3
Survivorship Function
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1000
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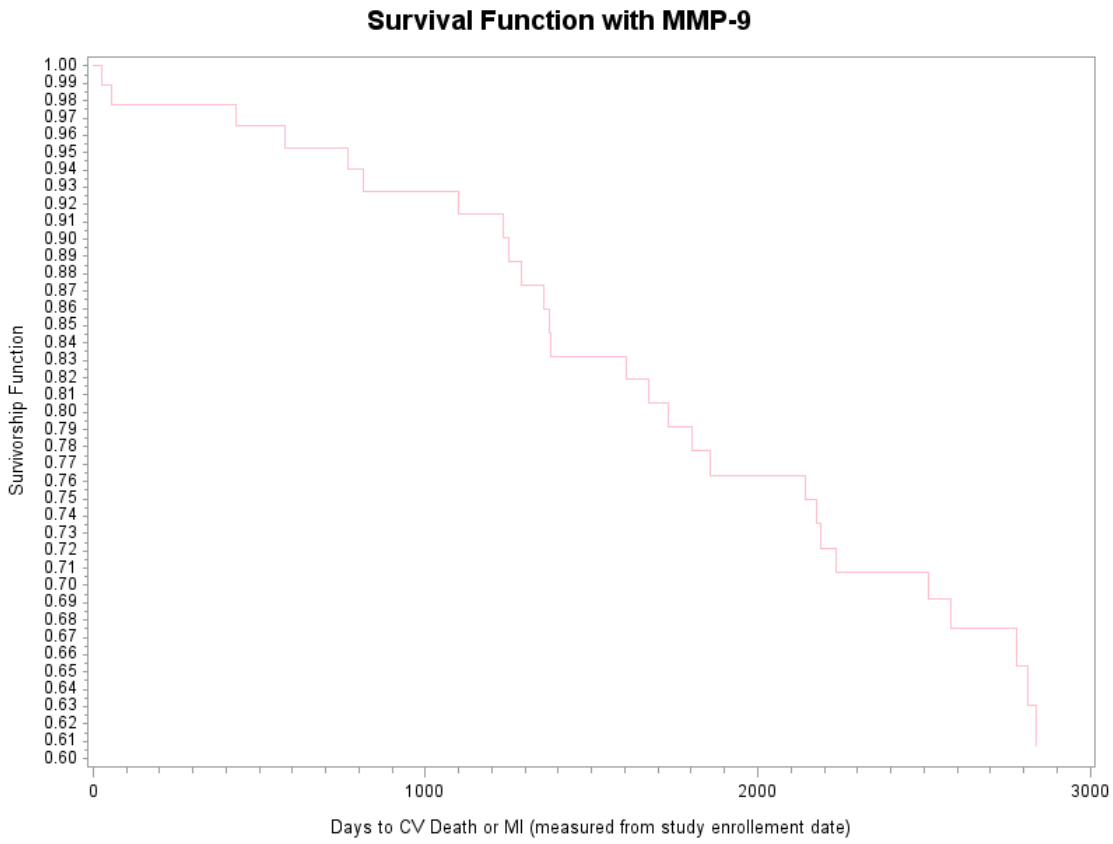
3000
Days to CV Death or MI (measured from study enrollement date)


Survival Function with MMP-9
Survivorship Function
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0.61
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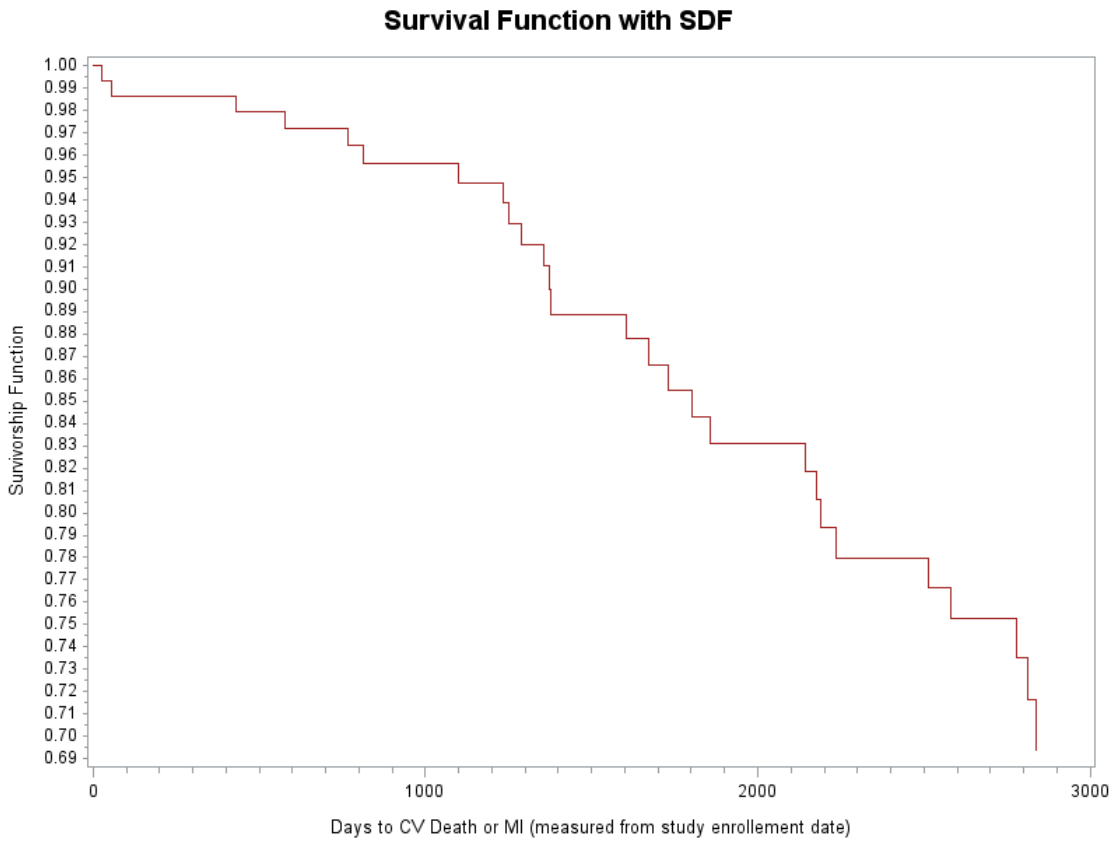
3000
Days to CV Death or MI (measured from study enrollement date)


Survival Function with SDF
Survivorship Function
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0.98
0.97
0.96
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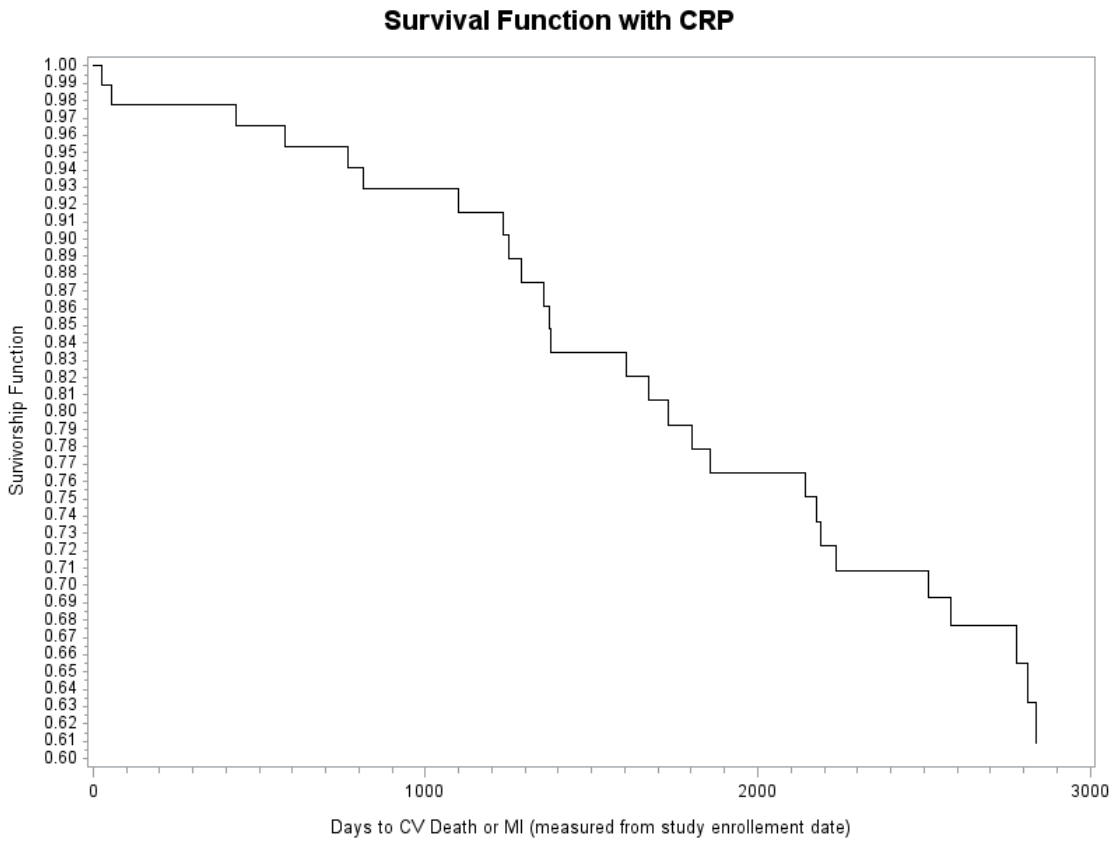
Days to CV Death or MI (measured from study enrollement date)


Survival Function with CRP
Survivorship Function
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1000
2000
3000
Days to CV Death or MI (measured from study enrollement date)