SUPPLEMENTAL MATERIAL

Expanded Methods

Clinical covariates associated with death/MI and death from prior modeling

Baseline clinical predictors were taken from Cox proportional hazard models developed from the overall MURDOCK CV study cohort that identified independent clinical variables associated with death and death/MI. The Cox models derived from the MURDOCK H1 CV study had C-indices of 0.792 and 0.752 for death and death/MI, respectively, and were internally validated using the bootstrap method with 200 bootstrapped samples for each model.

Correlation and use of the elastic net

A significant amount of correlation was found among the biomarkers (e.g., more than 30% of the pair-wise tests were found to be significant at α=0.05 after adjusting for multiple testing using the Bonferroni correction). The elastic net encourages grouping where a set of highly correlated predictors tends to be selected in or out of the model as a whole. Even though a few biomarkers failed to meet the linearity assumption required by the method, we chose to remain with the elastic net over other model selection methods because it most appropriately accounts for the substantial amount of correlation between the predictors.

Sensitivity analysis

Unlike the elastic net, the group LASSO performs selection on a group of related covariates (e.g., a set of spline covariates) as a whole rather than individually. This guarantees that all members of the group will be selected in or out of the model together, a feature not guaranteed

by the elastic net. Although the group LASSO allows us to fully account for all nonlinearity in the data when performing variable selection, a limitation is that it tends to select a single predictor, where the predictors could be an individual covariate or a group of related covariates, arbitrarily from a cluster of highly correlated variables. As a result, the group LASSO will tend to yield smaller models than the elastic net, which tends to select sets of correlated variables together.

Expanded Results

Linearity of biomarkers

For death, significant nonlinearity persisted for serum amyloid A (SAA), hsCRP, P-selectin, monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor alpha (TNFα), IL-6, and D-Dimer. Similarly, for death/MI, significant nonlinearity persisted after log-base-2 transformation for P-selectin, sFlt-1, MCP-1, TNFα, IL-10, von Willebrand Factor (vWF), and Lp-PLA2 (PLAC® and CAM).

Sensitivity analysis

In Model 1, the elastic net selected MMP-3, NT-proBNP, IL-6, IGFBP2, and D-Dimer as strongly associated with death, whereas only VCAM-1, RANTES, and NT-proBNP were strongly associated with death using the group LASSO; VCAM-1 was moderately associated with death in the elastic net, and IL-6 and IGFBP2 were moderately associated with death in the group LASSO. For death/MI, ICAM-1, MMP-3, NT-proBNP, IL-6, sCD40L, and IGFBP2 were strongly associated with outcomes using the elastic net, whereas IL-18, RANTES, NT-proBNP,

and IGFBP2 were strongly associated using the group LASSO; IL-6 was moderately associated with death/MI using the group LASSO.

Supplemental Table 1: Proteins Measured in MURDOCK H1 CV Study

Pathway	Protein	Platform	Lower Limit of Quantification	Upper Limit of Quantification	Intra-assay CV (%)
Inflammatory	TNF-α	Meso Scale Discovery	0.61 pg/mL	10,000 pg/mL	12.0
	TNF-β	MilliPlex	16 pg/mL	10,000 pg/mL	2.5
	hsCRP	Meso Scale Discovery	0.13 ng/mL	1000 ng/mL	3.9
	SAA	Meso Scale Discovery	0.13 ng/mL	1000 ng/mL	5.0
	MCP-1	Meso Scale Discovery	0.61 pg/mL	10,000 pg/mL	11.0
	IL-1β	Meso Scale Discovery	0.61 pg/mL	10,000 pg/mL	22.0
	IL-1α	Meso Scale Discovery	0.61 pg/mL	2500 pg/mL	NA
	IL-1RA	Meso Scale Discovery	24 pg/mL	100,000 pg/mL	2.0
	IL-2	Meso Scale Discovery	0.61 pg/mL	10,000 pg/mL	24.0
	IL-4	Meso Scale Discovery	0.61 pg/mL	10,000 pg/mL	32.0
	IL-6	Meso Scale Discovery	0.61 pg/mL	10,000 pg/mL	17.0
	IL-10	Meso Scale Discovery	1.52 pg/mL	10,000 pg/mL	18.0
	IL-18	Meso Scale Discovery	0.61 pg/mL	2500 pg/mL	4.0
	sICAM-1	Meso Scale Discovery	0.13 ng/mL	1000 ng/mL	3.7
	VCAM-1	Meso Scale Discovery	0.13 ng/mL	1000 ng/mL	3.7
	ICAM-3	Meso Scale Discovery	0.13 ng/mL	1000 ng/mL	11.0
	MCSF	Meso Scale Discovery	0.61 pg/mL	10,000 pg/mL	9.0
	G-CSF	Meso Scale Discovery	0.61 pg/mL	10,000 pg/mL	8.0
	sCD40L	MilliPlex	16 pg/mL	10,000 pg/mL	12.8
	P-selectin	Meso Scale Discovery	0.13 ng/mL	1000 ng/mL	8.0
	E-selectin	Meso Scale Discovery	0.13 ng/mL	1000 ng/mL	10.0
	Myeloperoxidase	MilliPlex	0.07 ng/mL	50 ng/mL	21.4
	Chemokine ligand 5 (aka RANTES)	MilliPlex	41 pg/mL	10,000 pg/mL	8.1
	Lp-PLA2 (PLAC®)	diaDexus	0.34 ng/mL		9.2
Thrombosis	Thrombomodulin	Meso Scale Discovery	0.13 ng/mL	1000 ng/mL	8.0
	PAI-1 (total)	MilliPlex	0.02 ng/mL	50 ng/mL	7.9
	Fibrinogen	MilliPlex	0.06 ng/mL	250 ng/mL	6.0
	vWF	American Diagnostics	5 ng/mL	100 ng/mL	7.0
	tPA	Assay Pro	7.8 pg/mL	500 pg/mL	15.0

	D-dimer	American Diagnostics	7.76 ng/mL	24.66 ng/mL	7.0
Vascular/endothelial	sFlt-1	Meso Scale Discovery	8.79 pg/mL	9000 pg/mL	10.0
	bFGF	Meso Scale Discovery	0.55 pg/mL	9000 pg/mL	21.0
	VEGF	Meso Scale Discovery	8.79 pg/mL	9000 pg/mL	13.0
	GDF-15	R&D (Quantikine)	23.44 pg/mL	1500 pg/mL	2.0
	PIGF	Meso Scale Discovery	2.20 pg/mL	9000 pg/mL	15.0
	PDGF AA	Meso Scale Discovery	16 pg/mL	10,000 pg/mL	10.2
	PDGF AB/BB	Meso Scale Discovery	41 pg/mL	10,000 pg/mL	20.5
Myocardial proteins	TnI	Meso Scale Discovery	0.01 ng/mL	80 ng/mL	7.0
	CKMB	Meso Scale Discovery	0.15 ng/mL	400 ng/mL	8.0
	Myoglobin	Meso Scale Discovery	19 ng/mL	3000 ng/mL	10.0
Extracellular matrix	MMP-1	Meso Scale Discovery	0.01 ng/mL	100 ng/mL	8.6
	MMP-3	Meso Scale Discovery	0.02 ng/mL	100 ng/mL	7.0
	MMP-9	Meso Scale Discovery	0.12 ng/mL	500 ng/mL	10.3
	TIMP-1	Meso Scale Discovery	54 pg/mL	40,000 pg/mL	4.3
Lipid-derived	Apolipoprotein A1	MilliPlex	0.08 ng/mL	1320 ng/mL	11.0
	Apolipoprotein B	MilliPlex	1.95 ng/mL	6080 ng/mL	23.0
	Apolipoprotein E	MilliPlex	0.05 ng/mL	720 ng/mL	14.0
Miscellaneous	OPGN	Meso Scale Discovery	4.3 pg/mL	200,000 pg/mL	5.4
	NT-proBNP	MilliPlex	16 pg/mL	50,000 pg/mL	35.0
	GH	MilliPlex	3.2 pg/mL	50,000 pg/mL	16.0
	IGFBP2	Thermo	5 ng/mL	3200 ng/mL	10-20
	PAPP-A	Alpco	0.5 ng/mL	30 ng/mL	NA
	LBP	Meso Scale Discovery	0.06 ng/mL	1000 ng/mL	11.0

Abbreviations: bFGF, basic fibroblast growth factor; CK-MB, creatine-kinase MB; *CV, cardiovascular; G-CSF*, g*ranulocyte colony-stimulating factor; GDF, growth differentiation factor; GH, growth hormone;* hsCRP, high-sensitivity C-reactive protein; *ICAM, intercellular adhesion molecule; IGFBP2,* insulin-like growth factor binding protein-2; *IL, interleukin; LBP; lipopolysaccharide-binding protein; Lp-PLA2, lipoprotein-associated phospholipase A2; MCP*, monocyte chemoattractant protein; *MCSF, macrophage colony stimulating factor; MMP, matrix metalloproteinase; NT-proBNP, N-terminal pro-B-type natriuretic peptide; OPGN, osteoprotegerin; PAI, plasminogen activator inhibitor; PAPP,* pregnancy-associated plasma protein; *PDGF, platelet-derived growth factor; PIGF, placental growth factor; RANTES, regulated on activation normal T cell expressed and secreted;* SAA, serum amyloid A; sCD40L, soluble CD40 ligand; *sFlt-1,* soluble Fms-like tyrosine kinase-1; sICAM-1, soluble intracellular adhesion molecule-1; *TIMP, tissue inhibitor of metalloproteinase; TNF-α, tumor necrosis factor; TnI, troponin I; tPA, tissue plasminogen activator; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factors; vWF, von Willebrand factor*

Supplemental Table 2: Biomarkers and Clinical Variables Associated with Death/MI in Group LASSO Models 1, 2, and 3

	% of 500 bootstrapped samples selected		
	Model 1	**Model 2***	**Model 3†**
Candidate biomarkers			
NT-proBNP	100	_‡	98.6
IL-18	98.0	92.0	97.6
IGFBP2	92.2	_	79.2
RANTES	88.8	76.0	88.4
IL-6	77.2	_	73.1
Clinical predictors			
RDW	NA	100	81.8
NYHA class	NA	100	73.7
Age (per 5 yrs)	NA	100	84.0
Ejection fraction (per 5% increase)	NA	100	75.4
Baseline Hgb (per 1 g/dL increase)	NA	100	83.8

*Model 2 was constrained to retain the following clinical variables predictive of death: age, sex, weight, blood pressure, heart rate, smoking history, diabetes, presence of chest pain at presentation, New York Heart Association class, ejection fraction, atrial fibrillation, left bundle branch block, corrected QT interval, red cell distribution width, serum blood urea nitrogen, creatinine, hemoglobin, white blood cell count, Duke coronary artery disease index, and Charlson comorbidity index.
†Model 3 allowed for selection of biomarkers and clinical variables.
‡Indicates protein selected in <70% of bootstrapped samples for the respective model.
Abbreviations: Hgb, hemoglobin; IGFBP2, insulin-like growth factor binding protein-2; IL, interleukin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; RANTES, regulated on activation normal T cell expressed and secreted; RDW, red cell distribution width

Supplemental Table 3: Biomarkers and Clinical Variables Associated with Death in Group LASSO Models 1, 2, and 3

	% of 500 bootstrapped samples selected		
	Model 1	**Model 2***	**Model 3†**
Candidate biomarkers			
NT-proBNP	100	_‡	98.2
RANTES	88.4	74.7	89.6
VCAM-1	87.4	_	83.4
IL-6	80.8	_	87.8
PDGF AB/BB	78.4	_	82.0
GDF-15	77.6	_	_
IGFBP2	70.7	_	_
Clinical predictors			
RDW	NA	100	94.8
NYHA class	NA	100	86.6
Age (per 5 yrs)	NA	100	76.4
Creatinine (per 0.1 mg/dL)	NA	100	72.3

*Model 2 was constrained to retain the following clinical variables predictive of death: age, sex, weight, blood pressure, heart rate, smoking history, diabetes, presence of chest pain at presentation, New York Heart Association class, ejection fraction, atrial fibrillation, left bundle branch block, left ventricular hypertrophy, corrected QT interval, red cell distribution width, serum sodium, BUN, creatinine, hemoglobin, white blood cell count, Duke CAD index, and Charlson comorbidity index.
†Model 3 allowed for selection of biomarkers and clinical variables.
‡Indicates protein selected in <70% of bootstrapped samples for the respective model.
Abbreviations: GDF-15, growth differentiation factor-15; IGFBP2, insulin-like growth factor binding protein-2; IL, interleukin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PDGF AB/BB, platelet-derived growth factor AA/BB; RANTES, regulated on activation normal T cell expressed and secreted; RDW, red cell distribution width; VCAM-1, vascular cell adhesion molecule-1