Protein Biomarkers and Cardiovascular Outcomes in People with Type 2 Diabetes and Acute Coronary Syndrome: The ELIXA Biomarker Study

Supplement

		Page
Supplemental Methods:	Identification and Analysis of Biomarkers	2
Table S1:	42 Biomarkers Included in the Analyses	4
Table S2:	Baseline Clinical Characteristics of the Biomarker Subgroup	5
Table S3:	Hazard Ratios of Biomarkers for the Composite MACE Outcome in 4957 ELIXA Participants	6
Table S4:	Hazard Ratios and 95% Confidence Intervals for Death from any Cause in 4957 ELIXA Participants	7
Table S5:	Hazard Ratios and 95% Confidence Intervals for Death from any Cause in 4957 ELIXA Participants Mortality	8
Table S6:	Models' Abilities to Predict Serious Health Outcomes	9
Figure S1:	Correlation of Standard and Multiplex NT-proBNP Assay	10

Supplemental Methods: Identification and Analysis of Biomarkers

Baseline serum from the 5182 ELIXA participants in the protein biomarker substudy was collected and stored as 2 aliquots (1.8 ml each) at -80C at Covance. Once the trial was completed, 1 aliquot was sent to Myriad RBM Inc. (Austin, Texas, USA) for multiplex analysis using the Luminex MAP (Multi-Analyte Profiling) technology. A panel of 49 protein biomarkers (Table S1) that are known to represent a diverse number of physiological processes related to cardiovascular disease and downstream effects including atherosclerosis, angiogenesis, extracellular matrix turnover, inflammation, tissue remodelling, adipocyte biology, kidney function/injury, endothelial dysfunction, calcification/mineral metabolism and myocyte stretch were analyzed[1,2]. The sample was sent without any phenotypic information to Myriad RBM Inc. and the results were returned to Sanofi and then forwarded to the ELIXA project office for subsequent statistical analyses.

Upon receipt of the levels of the biomarkers from Myriad RBM the data were scrutinized and three biomarkers (i.e., eotaxin 3, insulin-like growth factor 1, and kidney injury molecule 1) that were unable to be analyzed for technical reasons, and individuals for whom levels of all biomarkers were not available were removed from the database. When this process was completed, the final ELIXA biomarker database comprised 5176 people with values for each of 46 protein biomarkers. Two of these biomarkers (i.e., B-lymphocyte chemoattractant and macrophage colony-stimulating factor) were reported as non-detectable in ≥ 90% of participants and were not included in this analysis. For the remaining participants, if a particular biomarker was below the detection limit in a particular patient, the value assigned to that biomarker for that participant was the lower limit of detection. Finally, as 39% of ELIXA participants were using insulin, and as exogenous insulin affects endogenous C-peptide and insulin levels, these 2 biomarkers were also excluded leaving 42 biomarkers for analysis.

One of these 42 biomarkers was N-terminal prohormone B-type natriuretic peptide (NT-proBNP). Notably, levels of this protein measured using the multiplex system were highly correlated (Figure S1) with levels measured using the standard Immulite NT-proBNP assay at Covance Central Laboratory Services (Meyrin, Switzerland). Nevertheless, as all participants in the biomarker study were also included in analyses of NT-proBNP measured using the standard assay, and as articles reporting such analyses have previously been published[3,4], the values for NT-proBNP that were used in the analyses reported here are the baseline values that were measured using the standard (Immulite) assay and not those measured with the Myriad RBM assay. The 42 biomarkers that were analyzed therefore comprise the 41 that were measured by the Luminex technology at Myriad RBM Inc. plus the NT-proBNP levels that were measured previously.

Supplemental Results

A complete dataset of 42 protein biomarkers at baseline was available for 5112 ELIXA participants, 155 of whom (3.0%) had 1 or more missing data-points related to demographics, medications or cardiovascular risk factors. The final biomarker analyses were therefore done in 4957/6068 (81.7%)

participants. Compared to those not in the biomarker analyses, biomarker participants were frequently smokers, had a slightly lower cholesterol level and higher HbA1c, and were more likely to be on statins, antiplatelet drugs, and beta-blockers (Table S2). They included 3419 (69%) men of mean age 60 (10) years, of whom 11% were smokers, 77% had previous hypertension, 26% had albuminuria, 23% had previous heart failure. 93% were taking a statin, and 85% were taking an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker.

References

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2. Gerstein HC, Pare G, McQueen MJ, et al. Identifying Novel Biomarkers for Cardiovascular Events or Death in People With Dysglycemia. Circulation 2015;132(24):2297-304. DOI: 10.1161/CIRCULATIONAHA.115.015744.
3. Wolsk E, Claggett B, Pfeffer MA, et al. Role of B-Type Natriuretic Peptide and N-Terminal Prohormone BNP as Predictors of Cardiovascular Morbidity and Mortality in Patients With a Recent Coronary Event and Type 2 Diabetes Mellitus. J Am Heart Assoc 2017;29(6):6:e004743. DOI: 10.1161/JAHA.116.004743.
4. Wolsk E, Claggett B, Diaz R, et al. Increases in Natriuretic Peptides Precede Heart Failure Hospitalization in Patients With a Recent Coronary Event and Type 2 Diabetes Mellitus. Circulation 2017;136(16):1560-1562. DOI: 10.1161/CIRCULATIONAHA.117.029503.

Table S1: 42 Biomarkers Included in the Analyses			
Biomarkers	**Units**	**Biomarkers**	**Units**
Adiponectin	ug/mL	Interleukin-6 receptor	ng/mL
Alpha-1-Microglobulin	ug/mL	Leptin	ng/mL
Alpha-2-Macroglobulin	mg/mL	Macrophage-Derived Chemokine	pg/ml
Angiopoietin-2	ng/mL	Macrophage Migration Inhibitor Factor	ng/ml
Angiopoietin-like protein 2	ng/ml	Matrix Metalloproteinase-7	ng/mL
Apolipoprotein A-I	mg/mL	Neuropilin-1	ng/mL
Apolipoprotein B	ug/mL	Neutrophil Gelatinase-Associated Lipocalin	ng/mL
Apolipoprotein(a)	ug/mL	NT pro BNP*	pg/ml
Apolipoprotein E	ug/mL	Osteoprotegerin	pM
Chromogranin-A	ng/mL	Pentraxin 3	ng/ml
Cystatin-C	ng/mL	Peroxiredoxin-4	ng/mL
Factor VII	ng/mL	Selenoprotein P	ng/ml
Galectin-3	ng/mL	Sex Hormone-Binding Globulin	nmol/L
Gelsolin	ug/mL	Stromal cell-derived factor	pg/mL
Glucagon-like Peptide 1, total	pg/mL	Tamm-Horsfall Urinary Glycoprotein	ug/mL
Glutathione S-Transferase alpha	ng/mL	Tenascin-C	ng/mL
Growth/Differentiation Factor 15	ng/ml	Tissue Inhibitor of Metalloproteinases 1	ng/mL
Hepatocyte Growth Factor receptor	ng/mL	Tissue type Plasminogen activator	ng/mL
Insulin-like Growth Factor-Binding Protein 2	ng/mL	Trefoil Factor 3	ug/mL
Insulin-like Growth Factor Binding Protein 4	ng/mL	Vascular endothelial growth factor D	pg/mL
Interleukin-12 Subunit p40	ng/ml	YKL-40	ng/mL

*NT-proBNP levels were measured using standardized immunoassays. All other biomarkers were measured using the Myriad RBM multiplex assay system (Luminex)

Table S2: Baseline Clinical Characteristics of the Biomarker Subgroup			
	All Biomarker Participants (N=4957)	**Other Participants (N=1111)**	**P**
Men	3419 (69.0%)	788 (70.9%)	0.20
Age (years)	60.2 ± 9.7 years	60.6 ± 9.7 years	0.31
Men ≥55 years or Women ≥65 years	2958 (59.7%)	695 (62.6%)	0.08
Current Smoking	522 (10.5%)	187 (16.8%)	<0.001
Hypertension	3804 (76.7%)	831 (74.8%)	0.17
Micro/macroalbuminuria by history or lab	1281 (25.8%)	256 (25.1%)	0.61
Previous Myocardial Infarction	1130 (22.8%)	260 (23.4%)	0.66
Previous Heart Failure	1124 (22.7%)	234 (21.1%)	0.24
Systolic Blood Pressure	129.4 (17.4)	130.1 (16.7)	0.27
Estimated GFR	75.9 (21.2)	76.1 (21.8)	0.81
HbA1c	7.7 (1.3)	7.6 (1.2)	0.008
Total Cholesterol (mmol/L)	4.0 ± 1.1	4.1 ± 1.2	0.021
LDL Cholesterol (mmol/L)	2.0 ± 0.9	2.1 ± 1.0	0.010
HDL Cholesterol (mmol/L)	1.11 ± 0.28	1.14 ± 0.29	0.002
Baseline Meds			
Statin	4631 (93.4%)	996 (89.6%)	<0.001
ACE Inhibitor or ARB	4222 (85.2%)	934 (84.1%)	0.35
Antiplatelet drugs	4845 (97.7%)	1072 (96.5%)	0.016
Beta blockers	4213 (85.0%)	911 (82.0%)	0.013
Diuretics	1962 (39.6%)	434 (39.1%)	0.75
Any Insulin	1934 (39.0%)	440 (39.6%)	0.72
MACE during follow-up	630 (12.7%)	162 (14.6%)	0.09
Death during follow-up	349 (7.0%)	85 (7.7%)	0.48

Data are expressed as either N (%) or Mean (SD); ACE – angiotensin converting enzyme inhibitor; ARB – angiotensin receptor blocker

Table S3: Hazard Ratios of Biomarkers for the Composite MACE Outcome in 4957 ELIXA Participants						
Variables	**Clinical Risk Factors Model a**	**ELIXA BM + Clinical Model b**	**ORIGIN BM + Clinical Model c**	**NT-proBNP + Clinical Model d**	**NT-proBNP Alone Model e**	**ORIGIN[a] HRs Model f**
Clinical Risk Factors						
Albuminuria	1.68 (1.43, 1.97)	1.25 (1.06, 1.49)	1.16 (0.97, 1.38)	1.36 (1.15, 1.61)	X	1.07
Men	1.07 (0.89, 1.28)	1.34 (1.11, 1.62)	1.43 (1.18, 1.72)	1.21 (1.01, 1.45)	X	1.45
Male ≥55 / Females ≥65	1.77 (1.47, 2.13)	1.32 (1.09, 1.61)	1.29 (1.06, 1.57)	1.50 (1.24, 1.80)	X	1.14
LDL/HDL	1.15 (1.07, 1.24)	1.17 (1.09, 1.27)	1.15 (1.04, 1.26)	1.17 (1.08, 1.26)	X	1.10
Current Smoking	1.21 (0.94, 1.55)	1.33 (1.04, 1.70)	1.32 (1.03, 1.69)	1.32 (1.03, 1.69)	X	1.45
Hypertension	1.37 (1.09, 1.71)	1.53 (1.22, 1.91)	1.51 (1.21, 1.89)	1.56 (1.25, 1.96)	X	1.18
Prior Heart Failure	1.40 (1.17, 1.66)	1.15 (0.97, 1.38)	1.13 (0.95, 1.35)	1.16 (0.97, 1.39)	X	X
Diuretic Use	1.60 (1.35, 1.88)	1.30 (1.10, 1.54)	1.26 (1.06, 1.50)	1.32 (1.12, 1.57)	X	X
Biomarkers						
NT-proBNP	X	1.54 (1.40, 1.68)	1.42 (1.29, 1.57)	1.61 (1.48, 1.76)	1.79 (1.66, 1.94)	1.29
Trefoil Factor 3	X	1.23 (1.12, 1.35)	1.20 (1.07, 1.35)	X	X	1.22
GDF 15	X	X	1.01 (0.90, 1.13)	X	X	1.19
Apolipoprotein B	X	X	1.06 (0.96, 1.17)	X	X	1.19
Angiopoietin-2	X	X	1.13 (1.03, 1.23)	X	X	1.17
Osteoprotegerin	X	?	1.15 (1.04, 1.28)	X	X	1.18
Alpha-2-Macroglobulin	X	X	1.09 (1.00, 1.20)	X	X	1.15
Hep Growth Factor Receptor[a]	X	X	0.89 (0.92, 0.97)	X	X	0.89
Glutathione S-Transferase α	X	X	0.99 (0.92, 1.08)	X	X	0.86
Chromogranin-A	X	X	0.95 (0.87, 1.04)	X	X	0.85

An X in a cell for a particular model means that the indicated biomarker was not assessed in the model. [a]hazard ratios for proteins from the ORIGIN biomarker study[2]; BM – biomarker; HR – hazard ratio; Hep – hepatocyte; Mphage - macrophage

Table S4: Hazard Ratios and 95% Confidence Intervals for Death from any Cause in 4957 ELIXA Participants						
Variables	**Clinical Risk Factors Model a**	**ELIXA BM + Clinical Model b**	**ORIGIN BM + Clinical Model c**	**NT-proBNP + Clinical Model d**	**NT-proBNP Alone Model e**	**ORIGIN[a] HRs Model f**
Clinical Risk Factors						
Albuminuria	2.09 (1.69, 2.59)	1.11 (0.88, 1.40)	1.00 (0.79, 1.27)	1.33 (1.06, 1.66)	X	0.97
Men	0.79 (0.62, 1.00)	1.30 (1.02, 1.66)	1.32 (1.02, 1.70)	1.08 (0.85, 1.36)	X	1.23
Male ≥55 / Females ≥65	2.27 (1.76, 2.93)	1.40 (1.07, 1.83)	1.33 (1.01, 1.75)	1.60 (1.24, 2.06)	X	1.37
LDL/HDL	1.20 (1.08, 1.33)	1.21 (1.10, 1.34)	1.15 (1.01, 1.32)	1.18 (1.07, 1.31)	X	1.09
Current Smoking	1.13 (0.81, 1.58)	1.45 (1.04, 2.03)	1.45 (1.03, 2.05)	1.43 (1.02, 2.00)	X	1.55
Hypertension	1.23 (0.92, 1.63)	1.32 (0.99, 1.75)	1.26 (0.95, 1.68)	1.38 (1.04, 1.84)	X	1.10
Biomarkers						
NT-proBNP	X	2.01 (1.78, 2.28)	1.78 (1.55, 2.05)	2.45 (2.18, 2.75)	2.60 (2.34, 2.90)	1.22
Trefoil Factor 3	X	X	1.16 (0.99, 1.36)	X	X	1.26
GDF 15	X	X	1.06 (0.91, 1.23)	X	X	1.29
Apolipoprotein B	X	X	1.09 (0.95, 1.25)	X	X	1.13
Angiopoietin-2	X	1.28 (1.15, 1.44)	1.26 (1.12, 1.42)	X	X	1.19
Osteoprotegerin	X	1.34 (1.18, 1.52)	1.21 (1.04, 1.39)	X	X	1.21
Alpha-2-Macroglobulin	X	X	1.24 (1.10, 1.41)	X	X	1.20
Hep Growth Factor Receptor[a]	X	X	0.84 (0.75, 0.93)	X	X	0.88
Glutathione S-Transferase α	X	X	1.00 (0.89, 1.11)	X	X	0.84
Chromogranin-A	X	X	0.93 (0.82, 1.05)	X	X	0.86
Tenascin C	X	X	1.18 (1.05, 1.32)	X	X	1.14
Selenoprotein P	X	X	0.93 (0.84, 1.04)	X	X	0.86
Mphage-derived chemokine	X	X	0.96 (0.86, 1.07)	X	X	0.84
YKL-40	X	X	1.08 (0.96, 1.21)	X	X	1.11
IGF-BP 2	X	X	1.05 (0.91, 1.21)	X	X	1.08

An X in a cell for a particular model means that the indicated biomarker was not included in the model. [a]hazard ratios for proteins from the ORIGIN biomarker study; BM – biomarker; HR – hazard ratio; Hep – hepatocyte; Mphage - macrophage

Table S5: Hazard Ratios and 95% Confidence Intervals for Death from any Cause in 4957 ELIXA Participants						
Variables	**Clinical Risk Factors Model a**	**ELIXA BM + Clinical Model b**	**ORIGIN BM + Clinical Model c**	**NT-proBNP + Clinical Model d**	**NT-proBNP Alone Model e**	**ORIGIN[a] HRs Model f**
Clinical Risk Factors						
Albuminuria	1.94 (1.57, 2.40)	1.11 (0.87, 1.40)	1.00 (0.78, 1.27)	1.32 (1.05, 1.65)	X	0.97
Men	0.91 (0.72, 1.15)	1.32 (1.04, 1.69)	1.33 (1.03, 1.71)	1.12 (0.88, 1.42)	X	1.23
Male ≥55 / Females ≥65	2.09 (1.62, 2.71)	1.40 (1.07, 1.83)	1.33 (1.01, 1.75)	1.60 (1.24, 2.07)	X	1.37
LDL/HDL	1.13 (1.02, 1.25)	1.18 (1.07, 1.31)	1.14 (1.00, 1.30)	1.15 (1.04, 1.28)	X	1.09
Current Smoking	1.24 (0.89, 1.73)	1.44 (1.03, 2.02)	1.44 (1.02, 2.02)	1.43 (1.02, 2.00)	X	1.55
Hypertension	1.00 (0.75, 1.33)	1.19 (0.89, 1.60)	1.16 (0.87, 1.55)	1.26 (0.94, 1.68)	X	1.10
Prior Heart Failure	2.04 (1.64, 2.56)	1.44 (1.14, 1.81)	1.39 (1.10, 1.76)	1.49 (1.19, 1.88)	X	X
Diuretic Use	1.88 (1.50, 2.37)	1.30 (1.03, 1.65)	1.31 (1.03, 1.66)	1.35 (1.07, 1.71)	X	X
Biomarkers						
NT-proBNP	X	1.87 (1.64, 2.12)	1.66 (1.44, 1.91)	2.22 (1.97, 2.50)	2.60 (2.34, 2.90)	1.22
Trefoil Factor 3	X	X	1.13 (0.96, 1.32)	X	X	1.26
GDF 15	X	X	1.06 (0.91, 1.24)	X	X	1.29
Apolipoprotein B	X	X	1.09 (0.95, 1.25)	X	X	1.13
Angiopoietin-2	X	1.26 (1.12, 1.41)	1.23 (1.10, 1.39)	X	X	1.19
Osteoprotegerin	X	1.33 (1.17, 1.51)	1.21 (1.04, 1.39)	X	X	1.21
Alpha-2-Macroglobulin	X	X	1.23 (1.08, 1.39)	X	X	1.20
Hep Growth Factor Receptor[a]	X	X	0.83 (0.74, 0.93)	X	X	0.88
Glutathione S-Transferase α	X	X	1.00 (0.90, 1.12)	X	X	0.84
Chromogranin-A	X	X	0.93 (0.82, 1.06)	X	X	0.86
Tenascin C	X	X	1.16 (1.03, 1.30)	X	X	1.14
Selenoprotein P	X	X	0.94 (0.84, 1.04)	X	X	0.86
Mphage-derived chemokine	X	X	0.96 (0.86, 1.07)	X	X	0.84
YKL-40	X	X	1.09 (0.97, 1.23)	X	X	1.11
IGF-BP 2	X	X	1.07 (0.93, 1.24)	X	X	1.08

An X in a cell for a particular model means that the indicated biomarker was not assessed in the model. [a]hazard ratios for proteins from the ORIGIN biomarker study[2]; BM – biomarker; HR – hazard ratio; Hep – hepatocyte; Mphage - macrophage

Table S6: Models' Abilities to Predict Serious Health Outcomes

	MACE		Mortality	
Components of the Model	**C Statistics[1]**		**C Statistics[a]**	
Model a. Clinical Risk Factors	0.63 (0.61, 0.65)		0.66 (0.63, 0.69)	
Model b. ELIXA BM + Clinical	0.70 (0.68, 0.72)		0.79 (0.76, 0.81)	
Model c. ORIGIN BM + Clinical	0.71 (0.69, 0.73)		0.80 (0.77, 0.82)	
Model d. NT-proBNP + Clinical	0.69 (0.67, 0.71)		0.78 (0.75, 0.80)	
Model e. NT-proBNP Alone	0.67 (0.64, 0.69)		0.76 (0.73, 0.79)	
Model f. Based on ORIGIN HRs	0.69 (0.67, 0.72)		0.78 (0.75, 0.80)	
Comparisons of Models	**P[1]**	**NRI**	**P[1]**	**NRI**
Model b *vs.* a	<0.001	0.15 (0.11, 0.19)	<0.001	0.26 (0.20, 0.31)
Model c *vs.* a	<0.001	0.16 (0.12, 0.21)	<0.001	0.26 (0.20, 0.32)
Model d *vs.* a	<0.001	0.13 (0.09, 0.17)	<0.001	0.24 (0.19, 0.30)
Model e *vs.* a	0.022	0.05 (-0.01, 0.11)	<0.001	0.20 (0.13, 0.25)
Model f *vs.* a	<0.001	0.11 (0.05, 0.17)	<0.001	0.23 (0.16, 0.28)
Model b *vs.* d	0.020	0.02 (0.00, 0.06)	0.07	0.04 (-0.00, 0.08)
Model c *vs.* d	0.007	0.04 (0.02, 0.08)	0.002	0.05 (0.01, 0.10)
Model f *vs.* d	0.92	-0.00 (-0.05, 0.04)	1.00	0.01 (-0.06, 0.05)

N/A – not available as hazard ratios from ORIGIN for the composite outcome of MACE or unstable angina were unavailable. Each model assessed the set of 42 ELIXA biomarkers. [a]Each model's performance is described using Harrell's C statistic and the P values shown above are comparing the C statistics and ae based on Somer's D statistic; NRI (Net Reclassification Improvement) statistics are estimated after classifying people into 4 categories of risk based on the predicted probabilities of 0.05, 0.10, and 0.20 for developing the outcomes during the first 2 years of follow-up

Figure S1: Correlation of standard and multiplex NT-proBNP assay