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

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Word Count:	1350
Tables:	1
References:	15
Running Head:	Protein Biomarkers for Cardiovascular Outcomes
Registration:	clinicaltrials.gov NCT01147250

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Abstract

Background: Protein biomarkers may identify people with type 2 diabetes at high risk of cardiovascular outcomes and death.

Methods: Bio-banked serum from 4957 ELIXA (Evaluating Lixisenatide in Acute Coronary Syndrome) participants was analyzed. Forward-selection Cox models identified independent protein risk factors for major adverse cardiovascular events (MACE) and death, that were compared to a previously validated biomarker panel.

Results: NT-proBNP and osteoprotegerin predicted both outcomes. In addition, trefoil factor 3 predicted MACE, and angiopoietin-2 predicted death (C = 0.70 and 0.79 respectively compared to 0.63 and 0.66 for clinical variables alone). These proteins had all been previously identified and validated. Notably, C statistics for just NT-proBNP plus clinical risk factors were 0.69 and 0.78 for MACE and death respectively.

Conclusion: NT-proBNP and other proteins independently predict CV outcomes in people with type 2 diabetes following acute coronary syndrome. Adding other biomarkers only marginally increased NT-proBNP's prognostic value.

Introduction

Type 2 diabetes increases the risk of cardiovascular outcomes and death, and protein biomarkers may facilitate prediction of these outcomes[1,2]. Previously validated biomarkers for these outcomes were assessed using baseline serum from the Evaluating Lixisenatide in Acute Coronary Syndrome (ELIXA) trial[3].

Research Design and Methods

In the ELIXA trial, 6,068 people with type 2 diabetes and a recent acute coronary syndrome were randomly assigned to either lixisenatide or placebo. During 25 months of median follow-up, 792 (13.1%) participants experienced a major adverse cardiovascular event (MACE) of non-fatal MI, non-fatal stroke, or cardiovascular death and 434 (7.2%) died from any cause, with no difference among assigned groups. The ELIXA trial was approved by local ethics boards and all participants provided written informed consent. A subset of 5182 participants consented to the storage of blood for future analysis of the cardiovascular biomarkers noted in Table S1.

Statistical Analysis

After natural log transformation of non-normally distributed biomarkers, and converting the 42 values for each participant to standardized values with a mean of 0 and a SD of 1, Cox models were used to identify the subset of biomarkers that predicted each of MACE and death. After forcing in previously validated risk factors[4] a forward selection approach was used to identify biomarkers that independently predicted each outcome at a Bonferroni-corrected P-value of 0.001 (~0.05/42) to account for the 42 comparisons.

The 10 biomarkers previously identified as independent predictors of MACE, and 15 biomarkers (comprising these 10 plus 5 more) previously identified and validated as independent predictors

of death in the Outcomes Reduction with an Initial Glargine Intervention (ORIGIN) trial, were also assessed by including just them in the Cox models and estimating their hazard ratios using ELIXA data. Whether accounting for a history of heart failure or diuretic use modified the findings was explored in a sensitivity analysis.

The ability of various models to predict these 2 outcomes was analyzed using Harrell's C statistic and compared using Somers' D statistic. Net reclassification Improvement (NRI) statistics were estimated after classifying people into 4 risk categories 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[5]. All analyses were performed using STATA (Version 16, College Station TX).

Results

Baseline characteristics of the 4957/6068 (81.7%) included in the analyses are noted in the supplement and Table S2. During a median follow-up period of 2.1 years, the incidence of MACE in the biomarker subset was 12.7% (6.3/100 person-years) and the incidence of all-cause death was 7.0% (3.3/100 person-years).

Hazard ratios for the relationship between each of the 6 clinical risk factors and MACE are shown in the Table (column a). After including these 6 risk factors in the Cox model as independent variables and then applying a forward selection approach to the 42 measured biomarkers, 3 protein biomarkers significantly (all $p < 0.001$) predicted MACE (Table, column b). These included NT-proBNP (HR per SD 1.54; 95%CI 1.41, 1.68), trefoil factor 3 (HR per SD 1.18; 95% CI 1.08, 1.29), and osteoprotegerin (HR per SD 1.18; 95% CI 1.08, 1.30). Forcing the other seven ORIGIN biomarkers into the model only slightly attenuated these hazard ratios

(Table, column c). After also accounting for previous heart failure and diuretic use in a sensitivity analysis, osteoprotegerin was no longer identified as an independent predictor (Table S3).

Adding the biomarker with the largest effect size for MACE (NT-proBNP) to the 6 clinical risk factors increased its adjusted hazard ratio per SD from 1.54 to 1.71 (95% CI 1.57, 1.85). This only slightly attenuated the hazard ratio of 1.79 (95% CI 1.66, 1.94) which was estimated with a univariable model that did not include any clinical risk factors (Table, columns d and e).

Similar findings were noted for total mortality with the exception that angiopoietin-2 was identified instead of trefoil factor 3 (Table S4). Notably, the risk associated with a 1 SD increase in the NT-proBNP level was greater for total mortality than for MACE outcomes (Tables 1 and S4). As with MACE, accounting for previous heart failure and diuretic use in a sensitivity analysis slightly attenuated the hazard ratios (Table S5).

Table S6 summarizes and compares the performance characteristics of these models for MACE and death. The lowest C statistics for all outcomes were noted for models that included only the clinical variables, and the highest C statistics were noted for models that also included the biomarkers that were either selected by the forward selection process using ELIXA data (Model b), or that had previously been identified in the ORIGIN trial (Model c). This is reflected in the NRI statistic which showed that compared to the clinical risk factors alone, Model b and c clearly improved the ability to predict MACE (NRI 0.15 [95%CI 0.11, 0.19] and 0.16 [95%CI 0.12, 0.21] respectively), and death (NRI 0.26 [95%CI 0.20, 0.31] and 0.26 [95%CI 0.20, 0.32] respectively). Notably, these two models were only marginally superior to the model with the clinical risk factors and just NT-proBNP for both MACE and death.

Discussion

After accounting for clinical risk factors, NT-proBNP, trefoil factor 3 and osteoprotegerin independently predicted MACE, and a previously validated MACE biomarker panel comprising these three plus seven additional biomarkers also predicted MACE. Furthermore, NT-proBNP, osteoprotegerin, and angiopoetin-2 independently predicted death, and a previously validated death biomarker panel comprising these three plus twelve additional biomarkers also predicted death. Finally, after accounting for the clinical risk factors, the ability of NT-proBNP alone to discriminate between individuals who did and did not experience either MACE or death was only marginally lower than that of models with NT-proBNP plus other biomarkers.

These findings further validate the set of previously identified ORIGIN biomarkers for both MACE and mortality and extend the relevance of these biomarkers to people with type 2 diabetes and acute coronary syndrome. They also highlight the previously reported clinical relevance of NT-proBNP levels in such individuals[6,7], and are consistent with evidence that NT-proBNP, a protein reflecting left ventricular diastolic pressure, is prognostic for cardiovascular outcomes and death[8,9].

In contrast to NT-proBNP, the pathophysiologic link between trefoil factor 3[10] and cardiovascular disease is not clearly elucidated. Nevertheless, such a link has now been confirmed in the ORIGIN, HOPE and ELIXA trials, as well as in the Apixaban for Reduction in Stroke and Other Thromboembolic Events trial[11]. Osteoprotegerin (OPG) is a soluble member of the tumor necrosis receptor superfamily that's been implicated in vascular calcification and atherosclerosis. Meta-analyses have linked it to death and incident CVD[12], and other analyses

have linked it to heart failure[13,14]. Finally, angiopoietin-2 is an endothelial cell protein that is released in response to inflammatory and angiogenic signals[15].

ELIXA validation of the ORIGIN biomarker models confirms the robustness of these multiplex-measured proteins as predictors of cardiovascular outcomes, either because their concentrations reflect biologic process causing the outcomes, or because changes in their concentration might attenuate the outcomes. The fact that only 630 MACE outcomes and 42 biomarkers were available limited the power to detect small effects or signals from other proteins. Nevertheless, these findings support further inquiry into the cardiovascular role of the identified biomarkers and the routine measurement of NT-proBNP to identify patients at highest risk for future cardiovascular events or death.

Acknowledgements

Funding: The ELIXA trial as well as the ELIXA biomarker substudy was funded by Sanofi.

Contributions: HCG wrote the first draft of the paper and BC did the statistical analyses. SH identified the biomarkers to be measured in stored blood and coordinated their biochemical analyses. All the authors researched the data and critically revised the paper. HCG is the guarantor of the study and made the final decision to submit and publish the manuscript.

Disclosures: HCG holds the McMaster-Sanofi Population Health Institute Chair in Diabetes Research and Care. He reports research grants from Eli Lilly and Company, AstraZeneca, Merck, Novo Nordisk, and Sanofi; honoraria for speaking from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Novo Nordisk, DKSH, Zuellig, Roche, and Sanofi; and consulting fees

from Abbott, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Novo Nordisk, Sanofi, Kowa, Pfizer, Hanmi and Viatris; SH is an employee and shareholder of Sanofi; BC reports consulting fees from Amgen, Boehringer-Ingelheim, Cardurion, Corvia, Myokardia, and Novartis; LK reports honoraria from lectures from AstraZeneca, Novo, Novartis and Boehringer Ingelheim; APM reports personal fees from Astra Zeneca, Novartis, and Bayer for participation in study committees, outside the present work; JJVM is supported by a British Heart Foundation Centre of Research Excellence Grant. He declares payments to his employer, Glasgow University, for his work on clinical trials, consulting and other activities for Alnylam, Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, BMS, Cardurion, Cytokinetics, Dal-Cor, GSK, Ionis, KBP Biosciences, Novartis, Pfizer, Theracos. He also reports personal lecture fees from Abbott, Alkem Metabolics, AstraZeneca, Eris Lifesciences, Hikma, Lupin, Sun Pharmaceuticals, Medscape/Heart.Org, ProAdWise Communications, S & L Solutions Event Management Inc, Radcliffe Cardiology, Servier, the Corpus, Translational Medical Academy, Web MD and (as Director) the Global Clinical Trial Partners Ltd (GCTP); MCR reports honoraria for consulting from Adocia, Anji, DalCor and Sanofi. JCT holds the Canada Research Chair in personalized and translational medicine and the Université de Montréal Pfizer-endowed research chair in atherosclerosis. He reports grants from Amarin, AstraZeneca, Ceapro, DalCor Pharmaceuticals, Esperion, Ionis, Novartis, Pfizer, RegenXBio and Sanofi; honoraria from AstraZeneca, DalCor Pharmaceuticals, HLS Pharmaceuticals, Pendopharm and Pfizer, and holds minor equity interest in DalCor Pharmaceuticals. MAP reports research grant support to his institution from Novartis; Consultant to: AstraZeneca, Boehringer Ingelheim and Eli Lilly Alliance, Corvidia, DalCor, GlaxoSmithKline, Lexicon, NHLBI CONNECTs (Master Protocol Committee), Novartis, Novo

Nordisk, Peerbridge and Sanofi; and has equity in DalCor. KD and JLP have no relevant disclosures.

Table: 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	1.77 (1.51, 2.08)	1.19 (1.00, 1.42)	1.16 (0.97, 1.38)	1.38 (1.16, 1.63)	X	1.07
Men	0.98 (0.82, 1.18)	1.36 (1.13, 1.65)	1.41 (1.17, 1.71)	1.18 (0.98, 1.41)	X	1.45
Male ≥55 / Females ≥65	1.88 (1.56, 2.25)	1.29 (1.06, 1.56)	1.29 (1.06, 1.58)	1.51 (1.26, 1.82)	X	1.14
LDL/HDL	1.19 (1.11, 1.29)	1.19 (1.11, 1.29)	1.16 (1.05, 1.28)	1.19 (1.10, 1.29)	X	1.10
Current Smoking	1.16 (0.91, 1.49)	1.34 (1.05, 1.72)	1.32 (1.03, 1.70)	1.32 (1.03, 1.69)	X	1.45
Hypertension	1.54 (1.23, 1.92)	1.60 (1.29, 2.00)	1.59 (1.27, 1.99)	1.67 (1.34, 2.08)	X	1.18
Biomarkers						
NT-proBNP	X	1.54 (1.41, 1.68)	1.47 (1.34, 1.62)	1.71 (1.57, 1.85)	1.79 (1.66, 1.94)	1.29
Trefoil Factor 3	X	1.18 (1.08, 1.29)	1.22 (1.09, 1.36)	X	X	1.22
GDF 15	X	X	1.01 (0.90, 1.13)	X	X	1.19
Apolipoprotein B	X	X	1.07 (0.97, 1.18)	X	X	1.19
Angiopoietin-2	X	X	1.14 (1.04, 1.24)	X	X	1.17
Osteoprotegerin	X	1.18 (1.08, 1.30)	1.15 (1.04, 1.28)	X	X	1.18
Alpha-2-Macroglobulin	X	X	1.10 (1.00, 1.20)	X	X	1.15
Hep Growth Factor Receptor[a]	X	X	0.89 (0.82, 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, 1.05)	X	X	0.85

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

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