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

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OBJECTIVE

To use protein biomarkers to identify people with type 2 diabetes at high risk of cardiovascular outcomes and death.

RESEARCH DESIGN AND METHODS

Biobanked serum from 4,957 ELIXA (Evaluation of Lixisenatide in Acute Coronary Syndrome) trial participants was analyzed. Forward-selection Cox models identified independent protein risk factors for major adverse cardiovascular events (MACE) and death that were compared with 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 with 0.63 and 0.66 for clinical variables alone). These proteins had all previously been 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.

CONCLUSIONS

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

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 with use of baseline serum from the Evaluation of Lixisenatide in Acute Coronary Syndrome (ELIXA) (3).

RESEARCH DESIGN AND METHODS

In the ELIXA trial, 6,068 people with type 2 diabetes and recent acute coronary syndrome were randomly assigned to either lixisenatide or placebo. During median 25 months of follow-up, 792 (13.1%) participants experienced a major adverse cardiovascular event (MACE) of nonfatal MI, nonfatal stroke, or cardiovascular death and

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434 (7.2%) died of 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 5,182 participants consented to the storage of blood for future analysis of the cardiovascular biomarkers, noted in Supplementary Table 1.

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 an 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 ($\sim$0.05 of 42) to account for the 42 comparisons.

The 10 biomarkers previously identified as independent predictors of MACE, and the 15 biomarkers (comprising these 10 plus 5 more) previously identified and validated as independent predictors of death in the Outcome Reduction With 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 two outcomes was analyzed with Harrell C statistic and compared with Somers D statistic. Net reclassification improvement (NRI) statistics were estimated after classification of people into four 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 with STATA (version 16, College Station, TX).

RESULTS

Baseline characteristics of the 4,957 of 6,068 (81.7%) included in the analyses are noted in Supplementary Material and Supplementary Table 2. 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 (HRs) for the relationship between each of the six clinical risk factors and MACE are shown in Table 1 (model a). After inclusion of these six risk factors in the Cox model as independent variables and then application of a forward selection approach to the 42 measured biomarkers, three protein biomarkers significantly (all $P < 0.001$) predicted MACE (Table 1 [model b]). These included NT-proBNP (HR per SD 1.54; 95% CI 1.41, 1.68), trefoil factor 3 (1.18; 1.08, 1.29), and osteoprotegerin (1.18; 1.08, 1.30). Forcing the other seven ORIGIN Trial biomarkers into the model only slightly attenuated these HRs (Table 1 [model c]). After also accounting for previous heart failure and diuretic use in a sensitivity analysis, osteoprotegerin was no longer identified as an independent predictor (Supplementary Table 3).

Adding the biomarker with the largest effect size for MACE (NT-proBNP) to the six clinical risk factors increased its adjusted HR per SD from 1.54 to 1.71 (95% CI 1.57, 1.85). This only slightly attenuated the HR of 1.79 (1.66, 1.94), which was estimated with a univariable model that did not include any clinical risk factors (Table 1 [models d and e]).

Similar findings were noted for total mortality with the exception that angiopoietin-2 was identified instead of trefoil factor 3 (Supplementary Table 4). Notably, the risk associated with a 1-SD increase in NT-proBNP level was greater for total mortality than for MACE outcomes (Table 1 and Supplementary Table 4). As with MACE, accounting for previous heart failure and diuretic use in a sensitivity analysis slightly attenuated the HRs (Supplementary Table 5).

Supplementary Table 6 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 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 in comparisons with the clinical risk factors alone, with models b and c there was clearly improvement in the ability to predict MACE (NRI 0.15 [95% CI 0.11, 0.19] and 0.16 [0.12, 0.21], respectively) and death (0.26 [0.20, 0.31] and 0.26 [0.20, 0.32]). 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.

CONCLUSIONS

After clinical risk factors were accounted for, 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 3 plus 12 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 Trial 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 Trial, Heart Outcomes Prevention Evaluation (HOPE), and ELIXA trial, as well as in the Apixaban for Reduction in Stroke and other ThromboembolLic Events in atrial fibrillation (ARISTOTLE) trial (11). Osteoprotegerin (OPG) is a soluble member of the tumor necrosis receptor superfamily that has been implicated in vascular calcification and atherosclerosis. Meta-analyses have linked it to death and incident CVD (12), and

Table 1—HRs (with 95% CIs) of biomarkers for the composite MACE outcome in 4,957 ELIXA participants

	Clinical risk factors (model a)	ELIXA BM + clinical (model b)	ORIGIN Trial BM + clinical (model c)	NT-proBNP + clinical (model d)	NT-proBNP alone (model e)	ORIGIN Trial[a] (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, age ≥55 years, and female, age ≥65 years	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
BM						
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
GDF15	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
α-2-macroglobulin	X	X	1.10 (1.00, 1.20)	X	X	1.15
Hepatocyte growth factor receptor	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. BM, biomarkers. [a]HRs for proteins from biomarker study in the ORIGIN Trial.

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 Trial biomarker models confirms the robustness of these multiplex-measured proteins as predictors of cardiovascular outcomes, either because their concentrations reflect biologic processes 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.

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