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Date:
2024-11-05

Citation:
Stewart, R. A. H., Robledo, K. P., Tonkin, A. M., Keech, A., Kritharides, L., Marschner, I., Janus, E., Thompson, P. L., Watts, G. F., Zeller, T., White, H. D. & Simes, J. (2024). Plasma Protein Biomarkers and Long-Term Cardiovascular Mortality Risk in Patients With Chronic Coronary Heart Disease. Journal of the American Heart Association, 13 (21), pp.e034367-. https://doi.org/10.1161/JAHA.123.034367.

Persistent Link:
https://hdl.handle.net/11343/359110

Journal of the American Heart Association

ORIGINAL RESEARCH

Plasma Protein Biomarkers and Long-Term Cardiovascular Mortality Risk in Patients With Chronic Coronary Heart Disease

Ralph A. H. Stewart, MD; Kristy P. Robledo, PhD; Andrew M. Tonkin, MD; Anthony Keech, MD; Leonard Kritharides, MD; Ian Marschner, MD; Edward Janus, MD, PhD; Peter L. Thompson, MD; Gerald F. Watts, MD; Tanja Zeller, PhD; Harvey D. White, DSc; John Simes, MD

BACKGROUND: Protein biomarkers that reflect different pathophysiological pathways have been associated with the risk of adverse cardiovascular events. However, it is uncertain whether these associations are sustained with increasing years after the biomarkers are measured.

METHODS AND RESULTS: In this cohort study, 7745 patients with coronary heart disease who participated in the LIPID (Long-Term Intervention With Pravastatin in Ischemic Disease) trial, BNP (B-type natriuretic peptide), troponin I, cystatin-C, C-reactive protein, D-dimer and midregional proadrenomedullin were measured at baseline and after 1 year. Discrimination of plasma biomarker concentrations for cardiovascular death were evaluated in landmark analyses from 1 year for the next 5 years of the randomized trial, and for 10 additional years after trial completion. All 6 biomarkers were associated with risk of cardiovascular death (n=1903) both during and after the clinical trial (each $P<0.001$). C-statistics for BNP were 0.706 and 0.704; cystatin-C, 0.686 and 0.693; troponin I, 0.686 and 0.689; C-reactive protein, 0.655 and 0.684; D-dimer, 0.670 and 0.679, and midregional adrenomedullin, 0.686 and 0.688, respectively. In multivariable models, adding all 6 biomarkers to models with clinical risk factors increased the C-statistic for cardiovascular death from 0.709 to 0.775 during the clinical trial, and from 0.713 to 0.751 during 10-year follow-up after the randomized trial ($P<0.001$ for both).

CONCLUSIONS: In patients with chronic coronary heart disease, biomarkers that reflect different pathophysiological pathways are associated with the risk of cardiovascular death for at least the next 15 years.

Key Words: biomarkers ■ cardiovascular risk ■ coronary heart disease ■ death ■ long-term survival

Plasma concentrations of BNP (B-type natriuretic peptide), troponins, cystatin-C, CRP (C-reactive protein), and D-dimers are commonly measured to diagnose and manage acute cardiac and other medical conditions. These biomarkers, which reflect different pathophysiological pathways, have also been associated with the risk of future cardiovascular events.[1–5] Despite this, plasma protein biomarkers are not currently widely used to improve the accuracy of cardiovascular risk prediction. This is partly because some studies have suggested that adding individual biomarkers to clinical risk scores only modestly improves risk prediction.[6–9] In addition, for long-term cardiovascular risk assessment, it is possible but not certain whether associations between plasma biomarker concentrations and adverse cardiovascular events diminish with increasing time after the biomarkers are measured.[10]

In the LIPID (Long-Term Intervention With Pravastatin in Ischemic Disease) study plasma concentrations of BNP, troponin I, cystatin-C, CRP, D-dimers, and

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This manuscript was sent to Tochukwu M. Okwuosa, DO, Associate Editor, for review by expert referees, editorial decision, and final disposition.

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.123.034367

For Sources of Funding and Disclosures, see page 9.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- In patients with chronic coronary heart disease associations between the plasma concentrations of 6 biomarkers and the risk of cardiovascular death were sustained for at least 15 years after the biomarkers were measured.
- Adding several biomarkers that reflect different pathophysiological pathways to clinical risk models improved prediction of the long-term cardiovascular mortality rate.

What Are the Clinical Implications?

- Improved discrimination of long-term cardiovascular mortality risk may be useful to identify patients more likely to benefit from some preventive treatments.

Nonstandard Abbreviations and Acronyms

LIPID	Long-Term Intervention With Pravastatin in Ischemic Disease

midregional proadrenomedullin were associated with both fatal and nonfatal adverse cardiovascular events in patients with coronary heart disease (CHD) during the randomized clinical trial.[11,12] In this study, we determined whether associations of these biomarkers with cardiovascular death were maintained or decrease during 10 additional years of follow-up after completion of the clinical trial. We also evaluated whether risk models that include several biomarkers, and repeat biomarker measurements at 1 year, improve discrimination for cardiovascular death compared with clinical risk models without biomarkers.

METHODS

Ethics Approval

The LIPID trial, biomarker substudy, and long-term follow-up for clinical outcomes were approved by the ethics committee at each participating center. All patients gave written informed consent for the trial and separately for collection and analysis of biomarkers, and for long-term clinic or remote follow-up, and use of relevant state and national administrative medical data in Australia or New Zealand.

Study Population

Participants in the LIPID study were aged 31 to 75 years, had a hospital admission for acute myocardial infarction or unstable angina 3 to 36 months previously, and a total cholesterol of 155 to 271 mg/dL (4.0–7.0 mmol/L) and triglyceride levels <445 mg/dL (5.0 mmol/L). Patients with a known left ventricular ejection fraction <35% or with New York Heart Association class 3 or 4 heart failure symptoms were excluded. After informed consent and baseline assessment, patients were randomized to pravastatin 40 mg or placebo once daily. Randomization was from June 1990 to December 1992 and patients continued on randomized treatment until trial end in September 1997, a mean of 6.1 years. Descriptions of the design, conduct, and results of the LIPID study have been published previously.[13–15]

At randomization, patients were invited to participate in a "biomarker substudy," which included consent to store plasma samples taken at the baseline and 1-year follow-up visits for future analysis.[11] The population evaluated for this study was 7745 patients who were alive 1 year after randomization, who had measurement of biomarkers at baseline or at 1 year from stored samples, and who gave consent for long-term follow-up.

Measurement of Biomarkers

Blood samples had been obtained after a 12-hour fast into EDTA tubes. Plasma was then stored in freezers at –70 °C until analysis. Samples were analyzed centrally at the Multinational Monitoring of Trends and Determinants in Cardiovascular Disease biomarker laboratory, as previously reported.[11] BNP, troponin I, cystatin-C, CRP, D-dimer and midregional adrenomedullin were chosen for the current analysis because they were independently associated with fatal and nonfatal cardiovascular events during the clinical trial period, and reflect different pathophysiological pathways.[11] BNP was measured with a chemiluminescent immunoassay (ADVIA Centaur; Siemens Healthcare Diagnostics, Marburg, Germany) with an assay range of 2 to 5000 pg/nL and interassay coefficient of variation of 5.2%. Troponin I was measured using an ultra assay on an ADVIA Centaur XP system with an assay range of 0.006 to 500 ng/mL and coefficient of variation of 10% at 0.030 ng/mL. The upper reference limit based on 99% for the healthy population is 0.040 ng/mL. CRP, D-dimer, and cystatin C were measured by latex particle immunoassay (Architect c8000, Abbott Diagnostics, Chicago, IL). The assay range for CRP was 0.01 to 160 mg/dL; for D-dimer, 0 to 1600 ng/mL; and for cystatin C, 0.0005 to 10 mg/dL. The interassay coefficients of variation were 3.8% for CRP, 6.4% for D-dimer, and 1.2% for cystatin C. Midregional proadrenomedullin was analyzed by immunofluorescence assay (BRAHMS; Thermo Fisher Scientific, Waltham, MA) with an assay range of 0.05 to 10 nmol/L. The

functional assay sensitivity (20% cardiovascular interassay precision) was 0.25 nmol/L.[11]

Clincal Risk Factors

Clinical risk factors were assessed at the baseline assessment, and updated from recorded clinical data at the year 1 assessment. The ABC-CHD risk model, which was developed and validated in other chronic CHD cohorts,[16] was used to evaluate the contribution of major clinical risk factors to predictive models. Clinical risk factors in the ABC-CHD model include age, diabetes, current smoking, and peripheral artery disease, defined as history of stroke or claudication, and non–high-density lipoprotein cholesterol used in this analysis in place of LDL cholesterol. The LIPID risk model, which includes 21 risk factors and predicted fatal and nonfatal CHD events during the randomized phase of the LIPID trial, was also evaluated[17] (Table S1).

Primary Outcome

The primary end point was cardiovascular death. Associations of biomarkers with all-cause death are included in the appendix. During the trial, cause of death was determined by an outcomes adjudication committee, and during follow-up after trial completion from the Australian and New Zealand national death registries.[18] During the clinical trial period, cardiovascular cause of death determined from national registries had sensitivity of 92% and specificity of 90% for cardiovascular cause of death according to the adjudication committee who had access to all available clinical information.[19] Participants were censored when last known to be alive or at the date of death for another cause. Vital status and cardiovascular death were available to 2007, a maximum of 17 years after randomization.[18] Information on fatal cardiovascular events during long-term follow-up after trial completion is not currently available.

Statistical Analysis

Hazard ratios (HRs) for cardiovascular death during total follow-up were calculated for deciles of biomarker concentrations measured at baseline and at 1 year. Landmark models from 1 year were adjusted for age, sex, and randomization to pravastatin or placebo partitioned for trial and posttrial periods to meet proportional hazard assumptions. The fifth decile was chosen as the reference. Concentrations below the level of detection for the assay were allocated to the lowest deciles (for BNP, <2 pg/mL, n=625, 7%, midregional adrenomedullin <0.05nmol/L, n=125 (1.6%); and troponin I<0.006 ng/mL, n=2695, 33%). For troponin I, patients with plasma concentrations above the assay limit of detection were split into 6 groups with ≈11% in each group, with the lowest of these groups chosen as the reference group. Separate analyses were undertaken for biomarker concentration at baseline, at 1 year, and with both measurements included in models. For the primary analysis both baseline and 1-year biomarker concentrations were used. To evaluate associations with cardiovascular death for relatively higher and lower biomarker concentrations HRs are reported at the 25% and 75% compared to the median.

The discrimination of biomarker concentration for cardiovascular death were compared from 1 year to the end of the randomized clinical trial (median, 5 years), and from the end of the randomized clinical trial to the end of extended follow-up (median, 10 additional years). For this analysis, $\log_2$-transformed biomarker concentrations at baseline and 1 year were included in models. When associations between $\log_2$ biomarker concentration and cardiovascular death were nonlinear, a quadratic term was added. Statistical testing was by likelihood ratio tests, C-statistics, and integrated discrimination improvement index at 14 years,[20] adjusted for age, sex, and treatment group. Separate models included each biomarker, all 6 biomarkers, the ABC-CHD or LIPID risk models, and the 6 biomarkers added to each clinical risk model.

Data Access

Data that support the findings of this study will be made available upon reasonable request to Professor John Simes, LIPID study chair, National Health and Medical Research Council Clinical Trials Centre, University of Sydney, New South Wales, Australia (Email: john.simes@sydney.edu.au).

RESULTS

Description of Study Population

Clinical risk factors for the study population and for patients with and without cardiovascular death during total follow-up (average, 15 years) are presented in Table 1, and in Table S1 for additional clinical risk factors included in the LIPID risk model. Established clinical cardiovascular risk factors were associated with an increased HR for cardiovascular death. Plasma concentrations of the 6 biomarkers are presented in Table 2. On average, plasma concentrations of all 6 biomarkers at baseline and 1 year were similar. On average, plasma concentrations of all biomarkers were higher in patients who suffered a cardiovascular death during follow-up compared with those who did not (P<0.0001 for all; Table 2).

Table 1. Clinical Risk Factors of Study Population and by Occurrence of Cardiovascular Death During Follow-Up

Characteristic	Overall	Cardiovascular death	No cardiovascular death	HR for cardiovascular death (95% CI)	*P* value
Total follow-up	7745 (100)	1903 (25)	5842 (75)		
Clinical trial period					
Randomized to pravastatin	3883 (50)	236 (43)	3647 (51)	0.74 (0.62–0.87)	<0.001
Randomized to placebo	3862 (50)	319 (57)	3543 (49)		
Extended follow-up					
Randomized to pravastatin	3453 (51)	675 (50)	2778 (51)	0.97 (0.87–1.08)	0.61
Randomized to placebo	3301 (49)	673 (50)	2628 (49)		
Age in groups, y					
<60	1756 (23)	194 (10)	1562 (27)	1	<0.001†
60–64	2955 (38)	621 (33)	2334 (40)	2.11 (1.79–2.48)	
65–69	1886 (24)	577 (30)	1309 (22)	3.55 (3.01–4.17)	
70+	1148 (15)	511 (27)	637 (11)	6.09 (5.16–7.19)	
Male sex	6423 (83)	1577 (83)	4846 (83)	1.20 (1.06–1.35)	0.003
Clinical risk factors in ABC-CHD risk score					
Smoker,* n (%)	828 (11)	201 (11)	627 (11)	1.47 (1.27–1.71)	<0.001
Hypertension,* n (%)	3368 (43)	976 (51)	2392 (41)	1.33 (1.21–1.45)	<0.001
Diabetes, n (%)	654 (8)	255 (13)	399 (7)	1.93 (1.69–2.20)	<0.001
Non-HDL cholesterol, mean±SD‡	4.71±0.81	4.74±0.83	4.70±0.80	1.12 (1.06–1.19)	<0.001
Previous stroke*	345 (4.5)	139 (7.3)	206 (3.5)	1.69 (1.42–2.00)	<0.001
Claudication	760 (9.8)	267 (14)	493 (8.4)	1.51 (1.32–1.72)	<0.001

Results are number (% of group) unless otherwise indicated. See Table S1 for data on all clinical risk factors included in LIPID risk model. 95% CI for cardiovascular death, adjusted for randomized to pravastatin, sex, and age group. CHD indicates coronary heart disease; HDL, high-density lipoprotein; HR, hazard ratio; and LIPID, Long-Term Intervention With Pravastatin in Ischemic Disease.

*Denotes risk factors with status updated at 1 year.

†Denotes *P* value for trend calculated using the median age for each category from a Cox regression analysis.

‡Non-HDL cholesterol is measured in mmol/L.

Associations Between Biomarker Concentrations and Cardiovascular Death

HRs for cardiovascular death during total follow-up by population decile of biomarker plasma concentration at baseline are plotted in Figure 1 for BNP, troponin I, cystatin-C, CRP, D-dimer, and midregional adrenomedullin, adjusted for age, sex, and pravastatin treatment group. There were graded associations between population decile of plasma concentration of all 6 biomarkers and the risk of cardiovascular death. These associations were similar when measured at baseline and at 1 year (Figures S1 and S2).

Associations with cardiovascular death for $\log_2$-transformed biomarker concentration were similar for measurements made at baseline and 1 year, but stronger in models that included both baseline and 1-year biomarker concentrations (Table S2). All 6 biomarkers were also associated with the risk of all-cause and non-cardiovascular death (Table S3).

The risk of cardiovascular death by concentration of biomarker compared with the population median are displayed in Table 3. For all biomarkers, the HR for cardiovascular death during total follow-up was lower when the biomarker concentration was less than the median population concentration (reported for 25%), and higher when greater than median (reported for 75%) ($P<0.001$ for all). These models included $\log_2$-transformed baseline and 1-year biomarker concentration, a quadratic term for BNP, midregion adrenomedullin, and D-dimer, and were adjusted for age, sex, and pravastatin treatment group.

Comparison of Different Clinical and Biomarker Risk Models

Models that included baseline and 1-year concentrations of all 6 biomarkers improved prediction of cardiovascular mortality risk compared with individual biomarkers separately and compared with the clinical risk scores without biomarkers, indicated by higher C-statistics for cardiovascular death and higher integrated discrimination improvement index with nonoverlapping 95% CIs (Table 4).

BNP was the biomarker with the best discrimination for cardiovascular mortality risk. Stepwise addition of

Table 2. Plasma Concentrations of 6 Protein Biomarkers at Baseline and Year 1 by Occurrence of Cardiovascular Death During Follow-Up

Biomarker	Overall, N=7745	Cardiovascular death, N=1903	No cardiovascular death, N=5842
BNP, pg/mL			
Baseline	23 (10–50)	37 (16–80)	20 (9–41)
Year 1	20 (7–45)	36 (15–75)	17 (6–37)
Troponin I, ng/mL			
Baseline	0.010 (0.006–0.020)	0.014 (0.006–0.026)	0.009 (0.006–0.019)
Year 1	0.009 (0.006–0.019)	0.013 (0.006–0.026)	0.008 (0.006–0.017)
Troponin I less than detectable limit, n (%)*			
Baseline	2948 (38)	525 (28)	2423 (41)
Year 1	2751 (41)	485 (30)	2266 (45)
Cystatin-C, mg/dL			
Baseline	0.81 (0.72–0.92)	0.88 (0.77–1.01)	0.79 (0.71–0.90)
Year 1	0.81 (0.72–0.92)	0.87 (0.77–1.02)	0.79 (0.71–0.89)
C-reactive protein, mg/L			
Baseline	2.4 (1.2–4.7)	3.1 (1.5–6.1)	2.2 (1.1–4.4)
Year 1	2.3 (1.2–4.7)	2.9 (1.5–5.8)	2.2 (1.1–4.4)
D-Dimer, ng/mL			
Baseline	171 (111–271)	205 (136–326)	162 (104–255)
Year 1	172 (111–274)	210 (136–337)	161 (105–254)
Midregional proadrenomedullin, nmol/L			
Baseline	0.47 (0.38–0.58)	0.53 (0.42–0.65)	0.46 (0.37–0.55)
Year 1	0.47 (0.37–0.58)	0.53 (0.42–0.65)	0.45 (0.36–0.56)

Results are median (interquartile range). BNP indicates B-type natriuretic peptide.
*For troponin I, the number and percentage of patients below the limit of detection is given.

BNP, troponin I, cystatin-C, CRP, and D-dimer incrementally improved risk prediction in models that also included age, sex, treatment group, and CHD clinical risk markers (C-statistics, 0.716, 0.724, 0.733, 0.736, and 0.738, respectively), but midregional adrenomedullin did not further improve risk prediction when added after the other 5 biomarkers (C-statistic, 0.738; Table S4). The C-statistic for cardiovascular death decreased when estimated glomerular filtration rate estimated using serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration formula was substituted for cystatin-C in equivalent models (C-statistic, 0.726 versus 0.733; Table S4).

Models that combined clinical risk factors, 5 or 6 biomarkers, age, sex, and treatment group had the best discrimination for cardiovascular mortality risk (Table 4).

Comparison of Cardiovascular Risk Prediction During the Clinical Trial and Late Follow-Up

Discrimination for cardiovascular death for each of the biomarkers were similar during the clinical trial and late follow-up periods (Table 4). HRs for cardiovascular death with increasing plasma concentrations of each of the 6 biomarkers during the clinical trial and during follow-up after the trial are displayed in Figure 2. Cardiovascular mortality risk prediction from models with 6 biomarkers, and from combined biomarker and clinical risk models were also similar during the clinical trial and during late follow-up after the trial (Table 4).

DISCUSSION

In patients with chronic CHD, BNP, troponin I, cystatin-C, D-dimer, CRP, and midregional proadrenomedullin independently improved prediction of cardiovascular mortality risk during long-term follow-up. Risk discrimination was further improved when all 6 of these biomarkers were included in risk models and when biomarkers were added to clinical risk factors. Risk discrimination was similar for the first ≈5 years after the biomarkers were measured and for the next 10 years after completion of the clinical trial. Therefore, associations between plasma concentration of each of these biomarkers and cardiovascular mortality risk are sustained for many years after they are measured.

Plasma concentrations of BNP increase with myocyte stress and myocardial dysfunction, troponin I with myocardial injury or cell death, CRP with inflammation, D-dimer with thrombosis, cystatin-C with renal dysfunction, and midregional proadrenomedullin with

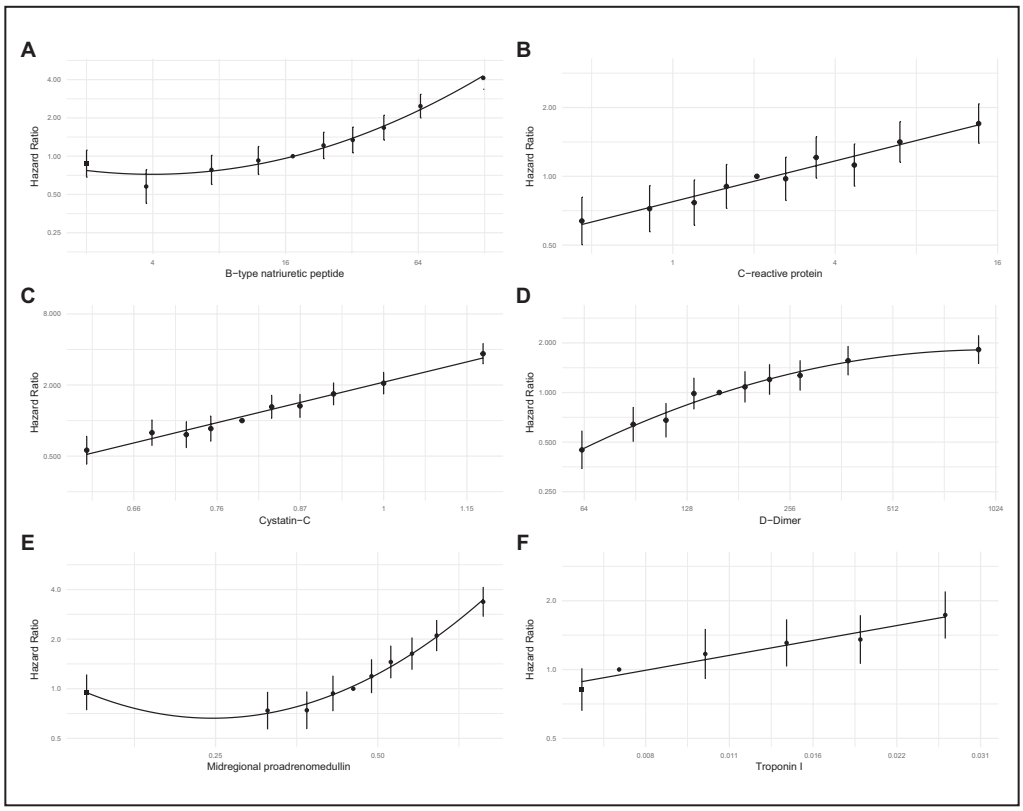

Figure 1. Relationship between the most recent biomarker concentration in deciles and cardiovascular death during the next 15 years.
Relationship with cardiovascular death over deciles of year 1 biomarkers on $\log_2$ scale, in a landmark analysis at 1 y for (**A**) B-type natriuretic peptide in pg/mL, (**B**) C-reactive protein in mg/L, (**C**) cystatin-C in mg/dL (**D**) D-dimer in ng/mL, (**E**) midregional proadrenomedullin in mmol/L, and (**F**) troponin I in ng/mL. Censored data are given with square boxes, and circles give measured data. For troponin I ≈33% of data fall below the detectable limit. The first square denotes all censored data, and the other 5 circles each denote ≈11% of the measured data.

changes in vascular and myocardial function. Although these 6 biomarkers provide information on different pathophysiological pathways, there were several similarities in their association with cardiovascular death. For all biomarkers there were graded increases in cardiovascular mortality risk with increase in plasma biomarker concentration both below and above normal reference ranges, and these associations were sustained for many years. Using the full range of biomarker concentrations therefore provides more information than a "threshold" concentration to define lower- and higher-risk categories.[8,9]

Biomarkers measured following an acute cardiovascular event are less predictive for cardiovascular mortality risk during long-term follow-up than measurements made several months after the acute event.[21,22] The observation that associations between biomarker concentration and cardiovascular death were sustained over many years suggests that they reflect long-term pathophysiology. However, even in clinically stable patients, there is apparently random variation in plasma concentrations of biomarkers over time. In this study including baseline and 1-year biomarker concentrations in models improved risk prediction compared with either measurement alone, consistent with the conclusion that ≥2 measurements provide a more reliable estimate of long-term "usual" concentrations.

In general, patients who have a higher absolute cardiovascular risk have a greater benefit from preventive treatment. In a secondary analysis for the LIPID trial, patients with higher plasma concentrations of each of the 6 biomarkers evaluated in this study had a greater absolute decrease in CHD death or nonfatal myocardial infarction with pravastatin treatment compared with placebo.[11] Clinical practice guidelines for cardiovascular disease prevention recommend absolute cardiovascular risk is estimated to guide treatment decisions. This study supports inclusion of several biomarkers in risk models with clinical risk factors to improve discrimination for long-term cardiovascular death.[23]

Table 3. Associations Between Plasma Concentrations of 6 Biomarkers and Cardiovascular Death During 15-Year Follow-Up

Biomarker	Biomarker concentration	Biomarker concentration at 25%	Cardiovascular death at 25%	Biomarker concentration at 75%	Cardiovascular death at 75%	Likelihood ratio test
	Median for population		HR (95% CI)*		HR (95% CI)*	*P* value†
BNP, pg/mL	21	7.6	0.7 (0.65–0.76)	46	1.18 (1.08–1.27)	<0.0001
C-reactive protein, mg/L	2.3	1.2	0.9 (0.86–0.94)	4.8	1.12 (1.08–1.17)	<0.0001
Cystatin-C, mg/dL	0.81	0.72	0.82 (0.77–0.88)	0.93	1.23 (1.17–1.3)	<0.0001
D-Dimer, ng/mL	175	113	0.9 (0.83–0.97)	279	1.16 (1.1–1.23)	<0.0001
Troponin I, ng/mL	0.010	0.006	0.88 (0.84–0.92)	0.020	1.11 (1.08–1.15)	<0.0001
Adrenomedullin, nmol/L	0.47	0.37	0.78 (0.75–0.82)	0.58	1.01 (0.95–1.08)	<0.0001

In all cases, the addition of the biomarker terms into the model indicates better goodness of fit. BNP indicates B-type natriuretic peptide; and HR, hazard ratio.

*The HRs with 95% CIs for cardiovascular death at the 25th and 75th centiles compared with the median biomarker concentration for the study population are reported for 6 biomarkers, adjusted for randomized to pravastatin, sex (reference female), and age group.

†Likelihood ratio tested a model without the biomarker terms (sex and randomized to pravastatin) vs a model with the baseline biomarker and the most recent biomarker values, plus the censoring indicators (if required) and the quadratic terms (if required).

Study Limitations

Cardiovascular death, rather than total death, was the primary outcome measure because it is more relevant for targeting cardiovascular preventive treatments. A limitation is that ascribing a cardiovascular cause of death from national registry data may be uncertain in

Table 4. Comparison of Different Models for Discrimination of Cardiovascular Death During Total Follow-Up, During the Randomized Clinical Trial (<5 Years) and During Late Follow-Up After the Clinical Trial (>5–15 Years)

Biomarker (s) and/or clinical risk factors included in model*	Total follow-up			During trial <5 y	Late follow-up >5–15 y
	IDI† at 14 y (95% CI)	*P* value	C-Statistic	C-Statistic	C-Statistic
Plasma protein biomarkers					
BNP	0.057 (0.046–0.071)	<0.001	0.702	0.706	0.704
Troponin I	0.027 (0.019–0.034)	<0.001	0.685	0.686	0.689
Cystatin-C	0.042 (0.032–0.055)	<0.001	0.688	0.686	0.693
C-reactive protein	0.023 (0.015–0.030)	<0.001	0.672	0.655	0.684
D-Dimer	0.016 (0.011–0.022)	<0.001	0.672	0.670	0.679
Midregion adrenomedullin	0.036 (0.028–0.047)	<0.001	0.684	0.686	0.688
All 6 biomarkers*	0.107 (0.094–0.122)	<0.001	0.731	0.745	0.730
ABC-CHD risk score variables					
Clinical risk variables only†	0.026 (0.019–0.034)	<0.001	0.674	0.649	0.687
Clinical risk variables† + BNP + troponin I	0.092 (0.081–0.110)	<0.001	0.724	0.727	0.728
Clinical risk variables + 6 biomarkers‡	0.120 (0.107–0.138)	<0.001	0.738	0.749	0.741
LIPID risk model variables					
All LIPID risk variables§	0.066 (0.058–0.080)	<0.001	0.706	0.709	0.713
All LIPID risk variables + 6 biomarkers§	0.141 (0.130–0.160)	<0.001	0.752	0.775	0.751

BNP indicates B-type natriuretic peptide; CHD, coronary heart disease; HDL, high-density lipoprotein; HR, hazard ratio; and LIPID, Long-Term Intervention With Pravastatin in Ischemic Disease.

*All models included age, sex, and pravastatin treatment group, stratified for the randomized clinical trial and late follow-up periods. Time is counted from the 1-year assessment. When a biomarker is included in the model, this includes values at baseline and most recent, as well as censoring indicator (if required) and quadratic terms (if required).

†Integrated discrimination improvement used to compare the strength of models for discrimination of cardiovascular death during total follow-up compared with a reference model, which included age, sex, and pravastatin treatment group. The integrated discrimination improvement index is not reported for the randomized clinical trial and late follow-up periods because values are not comparable between these periods.

‡ABC risk variables includes smoking status at 1 y, diabetes at baseline, non-HDL at baseline, and poly-vascular disease (claudication at baseline or stroke at 1 year).

§Lipid risk variables includes smoking status at 1 y, diabetes at baseline, non-HDL at baseline, stroke at 1 y, claudication at baseline, hypertension at 1 y, systolic blood pressure at 1 y, white blood cell count at 1 y, dyspnea at 1 y, angina grade at 1 y, and revascularization at 1 y; body mass index at baseline, index event myocardial infarction or unstable angina at baseline, HDL cholesterol at baseline, triglycerides at baseline, fasting glucose at baseline, and atrial fibrillation at baseline.

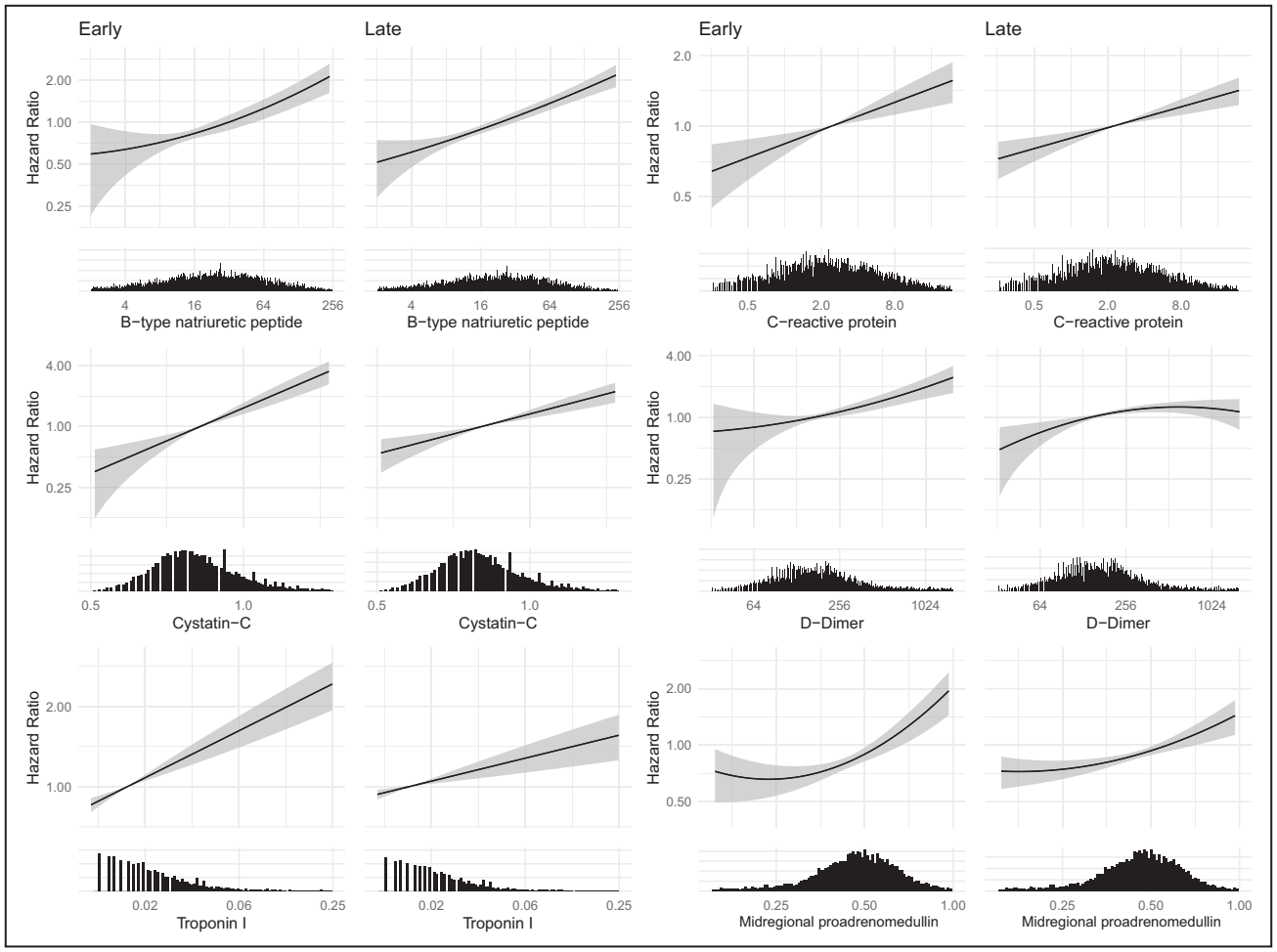

Figure 2. Hazard ratios for cardiovascular death for each biomarker during early and late follow-up after the clinical trial. Early follow-up refers to the randomized clinical trial period. Late follow-up refers to follow-up after the randomized trial. Hazard ratios for cardiovascular death by plasma concentration of 6 biomarkers, adjusted for age, sex, and randomized to pravastatin. The most recent biomarker measurement is used. Distributions shown on $\log_2$ scale underneath. **A**, B-type natriuretic peptide in pg/mL, (**B**) C-reactive protein in mg/L, (**C**) cystatin-C in mg/dL, (**D**) D-dimer in ng/mL, (**E**) midregional proadrenomedullin in mmol/L, and (**F**) troponin I in ng/mL. The distribution of plasma concentrations for each biomarker are displayed on the x-axis.

some patients.[19] Associations between all 6 biomarkers and all-cause death were similar or slightly weaker than those for cardiovascular death. Nonfatal cardiovascular events are relevant to cardiovascular risk prediction but were not available during late follow-up from the national mortality registries used for this study.

Lower accuracy in measurement of biomarker concentrations will decrease the strength of the observed compared with true association with cardiovascular mortality risk. The accuracy of measurement of biomarkers may have been influenced by handling of blood samples, prolonged storage, or the assay used. Some studies have reported a decrease in measured plasma BNP concentration with prolonged storage.[24] Collection of blood in citrated heparin is currently recommended for coagulation tests, but the D-dimer assay used in this study was validated for collection in EDTA.[25] Higher-sensitivity troponin I assays, compared with the ADVIA Centaur XP assay used in this study, are likely to improve risk prediction, especially between patients with lower troponin I concentrations.[26] Validation studies are needed to determine whether risk estimates from this study are similar in other populations, and for biomarkers measured as part of usual clinical care from fresh blood samples using different assays. Statistically strong graded associations between plasma concentrations of all 6 biomarkers and long-term cardiovascular death were observed despite these potential limitations. In this study, the same biomarker measurements were used to evaluate associations during the clinical trial and late follow-up periods, so possible bias related to biomarker measurement was the same for these comparisons.

Medical treatments could decrease the strength of association between biomarker plasma concentration

and long-term cardiovascular risk by decreasing the risk of adverse cardiovascular events and/or lowering the plasma concentrations of some biomarkers. In this study, associations were similar in pravastatin and placebo groups, but effects of other treatments could not be evaluated.

Left ventricular ejection fraction is an important risk factor for cardiovascular death and for targeting some treatments but was not collected in the LIPID trial. Biomarkers not measured in this study may also improve risk prediction. Further research is needed to develop risk models suitable for clinical use and to validate these in different populations. Advances in machine learning are likely to simplify inclusion of many variables in risk models.[27]

CONCLUSIONS

In stable patients with CHD, associations between plasma concentrations of BNP, troponin I, cystatin-C, CRP, D-dimers and midregional proadrenomedullin and the risk of cardiovascular death were sustained for at least ≈15 years after the biomarkers were measured. Except when influenced by acute disease, plasma concentrations of each of these biomarkers reflect long-term pathophysiology, which may be relatively stable over many years.

ARTICLE INFORMATION

Received March 11, 2024; accepted August 23, 2024.

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Acknowledgments

The authors acknowledge investigators, coordinators, patients, and relatives from participating centers for their long commitment to the LIPID study.

Sources of Funding

The LIPID Study (URL: https://anzctr.org.au; ACTRN12616000535471) was originally funded by Bristol-Myers Squibb (Princeton, NJ) and conducted under the auspices of the National Heart Foundation of Australia (Melbourne, Australia). This biomarker research was also supported by project grant 490968 from the National Health and Medical Research Council (NHMRC) of Australia. The design, management, analysis, and interpretation of this substudy were conducted independently of the study sponsor.

Disclosures

Dr White reports grant support paid to the institution and fees for serving on steering committees from the American Regent; DalCor Pharma UK, Inc; CSL Behring; the National Institutes of Health; Esperion Therapeutics Inc; Omthera Pharmaceuticals; and Sanofi Aventis Australia Pty Ltd. Dr Simes reports research funding support from Bayer, Bristol Myers Squibb, Astra Zeneca, Pfizer MSD, and Roche. Dr Kritharides reports honoraria for advisory board participation or speaker fees from Novartis, Amgen, and Seqiris. Dr Tonkin reports research funding support from Bayer and honoraria for advisory board participation or speaker fees from Amgen, Boehringer Ingelheim, Pfizer, and Sanofi. The remaining authors have no disclosures to report.

Supplemental Material

Tables S1–S4
Figures S1–S2

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