Midregional proadrenomedullin and its change predicts recurrent major coronary events and heart failure in stable coronary heart disease patients: The LIPID study

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Acknowledgement of grant support: The LIPID study was supported by a grant from the Bristol-Myers Squibb Pharmaceutical Research Institute and conducted under the auspices of the National Heart Foundation of Australia. The present biomarker study was supported by a Project Grant of the NHMRC of Australia.

Potential Conflicts of Interest: Malcolm West has received research grants from Merck Sharpe & Dohme and Astra Zeneca.

Stefan Blankenberg has received research funding from Boehringer Ingelheim, Bayer, Abbott Diagnostics, SIEMENS, Thermo Fisher and Roche Diagnostics and received honoraria for lectures or consulting from Boehringer Ingelheim, Bayer, Roche, Astra Zeneca, SIEMENS, Thermo Fisher and Abbott Diagnostics.

Harvey White has received research grants from Sanofi-Aventis; Eli Lilly; Medicines Company; NIH; Pfizer; Roche; Johnson & Johnson; Schering Plough; Merck Sharpe & Dohme; AstraZeneca; GlaxoSmithKline; Daiichi-Sankyo Pharma Development; Bristol-Myers Squibb and he has received a consultancy fee from Regado Biosciences.

Andrew Tonkin has received research funding or honoraria for lectures or consulting from Amgen, AstraZeneca, Boehringer Ingelheim, Genzyme, Merck Sharp and Dohme, Pfizer and Sanofi-Aventis/Regeneron.

All other authors do not report any conflicts of interest

Key words: Midregional proadrenomedullin, biomarker, risk factors, coronary heart disease, heart failure, LIPID study

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Structured Abstract:

Background: Biomarkers may contribute to risk stratification in coronary heart disease (CHD). We examined whether plasma midregional proadrenomedullin (MR-proADM) concentration at baseline and its change over one year predicts long-term outcomes in stable CHD patients.

Methods: The LIPID study randomised patients 3-36 months after an acute coronary syndrome with total cholesterol 4.0-7.0 mmol/L (155-271 mg/dl), to placebo or pravastatin 40 mg. Follow-up was 6.0 years. MR-proADM plasma concentrations at baseline and one year later were determined in 7,863 and 6,658 patients, respectively. These were categorised into quartiles to perform Cox regression analysis, adjusting for baseline parameters.

Results: Baseline MR-proADM concentrations predicted major CHD events (non-fatal myocardial infarction or CHD death; hazard ratio (HR) 1.52, 1.26–1.84 for Q4-Q1), CHD death (HR 2.21, 1.67-2.92), heart failure (HR 2.30, 1.78-2.97) and all-cause mortality (HR 1.82, 1.49-2.23). Associations were still significant after adjustment for baseline B-type natriuretic peptide (BNP) concentration. Increase in MR-proADM after one year was associated with increased risk of subsequent CHD events (HR 1.34, 1.08-1.66), non-fatal myocardial infarction (HR 1.50, 1.12-2.03), heart failure (HR 1.78, 1.37-2.30) and all-cause mortality (HR 1.31, 1.04-1.64). Associations with heart failure and all-cause mortality remained significant after adjusting for baseline and change in BNP concentration. Change in MR-proADM moderately improved risk reclassification for major CHD events (net reclassification improvement (NRI) 3.48%) but strongly improved risk reclassification for heart failure (NRI 5.60%).

Conclusions: Baseline and change in MR-proADM concentrations over one year are associated with risk of major clinical events, even after adjustment for BNP concentrations.

Introduction

Cardiovascular disease is the leading cause of death worldwide.[1] It is largely preventable, and biomarkers have received growing attention in attempts to improve the prediction of risk for atherothrombotic events.[2] Particularly, biomarkers reflecting cardiac volume or pressure overload appear promising in terms of risk stratification and prediction of subsequent CHD events and heart failure.[3, 4] Besides the natriuretic peptides[5], midregional proadrenomedullin (MR-proADM) has now moved into the centre of interest in this area. MR-proADM is a stable and surrogate marker for adrenomedullin (ADM) release.[6] ADM is a peptide hormone that acts as a vasodilator and plays important roles in the microcirculation and in endothelial dysfunction.[7, 8]

Plasma ADM concentrations are increased with myocardial infarction and correlate with the severity of associated heart failure.[9, 10] In patients with chronic heart failure, MR-proADM has been shown to be an independent predictor of mortality and to provide additional prognostic information beyond established biomarkers.[11-13] Increased MR-proADM concentrations have also been associated with a worse outcome in patients with acute dyspnoea and suspected heart failure.[14] However although MR-proADM is a powerful predictor of adverse outcomes in patients after myocardial infarction [15] and in CHD patients, [16] its importance in long-term follow-up of stable CHD patients, especially any association with incident heart failure, still needs further examination. Therefore, the aim of the present study was to assess the ability of MR-proADM to predict the risk of future major CHD events and heart failure in patients who were stable after previous myocardial infarction or unstable angina and also whether concentration changes over time translated into differences in risk of subsequent events. In both contexts, models adjusted for known prognostic variables including brain natriuretic peptide (BNP).

Methods

Study design and patients

The design and results of the Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) study has been described elsewhere.[17] Briefly, 9,014 patients with an acute myocardial infarction or hospitalisation for unstable angina 3-36 months previously were enrolled. Participants were aged between 31 and 75 years and were recruited from 87 centres in Australia and New Zealand. Baseline total cholesterol concentration was required to be 4-7 mmol/L (155-271 mg/dL) and fasting triglyceride concentration <5 mmol/L (445 mg/dL). Patients with a clinically significant medical or surgical event within the three months before study entry, current cardiac failure, renal or hepatic disease, or taking lipid-lowering agents were excluded from the study. Ejection fraction was not measured routinely prior to randomisation, but if this was documented to be <25%, such patients were also excluded. Coronary anatomy and whether or not patients had functional evidence of myocardial ischaemia was also unknown.

After an 8-week, single-blinded placebo run-in phase patients were randomised to either 40 mg pravastatin daily or matching placebo between June 1990 and December 1992. Both groups received dietary advice. Patients were followed-up for a median of 6.0 years. Vital status was ascertained in all but one patient.

Baseline data and a multivariate model were used to calculate a “global risk score” for each patient to rank the risk of CHD mortality or non-fatal myocardial infarction.[18] Total and high-density lipoprotein (HDL)-cholesterol concentrations, age, gender, smoking status, whether myocardial infarction or unstable angina was the qualifying event, previous coronary revascularisation procedures, diabetes mellitus, hypertension, and previous stroke were the independent significant predictors used to calculate the global risk score.

Laboratory methods

Biomarker measurements were available at baseline in 7,863 patients (6,530 male, 1,333 female). A total of 6,658 patients also had a MR-proADM assay available at one year after their randomisation to either pravastatin or placebo.

EDTA blood was drawn after a 12 hour fasting period. Samples were stored at –70 °C until final analyses. High density lipoprotein -cholesterol and triglyceride levels were measured directly. Low-density lipoprotein (LDL)-cholesterol was estimated indirectly, using the Friedewald formula.[19]

Biomarker samples were analysed in the MORGAM biomarker laboratory. MR-proADM was measured by an immunoluminometric assay (BRAHMS, Kryptor) with an assay range of 0.05-10 nmol/L. The functional assay sensitivity (20% CV interassay-precision) was 0.25 nmol/L. Precision data were estimated in accordance with CLSI (Clinical and Laboratory Standards Institute Guidelines) Guideline EP 5-A2 (Evaluation of Precision Performance of Clinical Chemistry Devices). Reference ranges for healthy individuals were 0.52 nmol/L for the 95% percentile and0.32 nmol/L for the median. The distribution of MrProADM was skewed, and also some values were below the lower limit at baseline and year 1. For these reasons and as pre-specified in the biomarker protocol, analyses were performed using quartiles.

BNP was measured using the sandwich-immunoassay/ADVIA Centaur BNP test kit (Siemens Healthcare) on an ADVIA Centaur XP with an assay range of 0-5000pg/ml. The interassay coefficient of variation was between 2.6 and 5.1%.

Biochemical analyses were performed blinded to randomised treatment.

Study outcomes

The primary pre-specified outcome for LIPID biomarker analyses was a composite of CHD death and non-fatal myocardial infarction (major CHD events). Additionally, endpoints for the present study included incident heart failure (hospitalisation or death from heart failure, as

diagnosed by ICD-9 codes (4280, 4281, 4289)), and all-cause mortality. Myocardial infarction was diagnosed by development of new pathological Q-waves of ≥0.03 seconds in at least 2 contiguous electrocardiographic leads or presence of at least two of the following: a history of typical ischaemic pain lasting for ≥15 minutes and unresponsive to sublingual nitrates; elevation of creatinine kinase MB-isoform >2 times the upper limit of normal; evolution of ST-T changes. All deaths, myocardial infarctions, and strokes were reviewed by Outcomes Assessment Committees whose members were blinded to treatment assignment. All analyses were pre-specified in a biomarker protocol. The trial and the biomarker analyses were conceived, managed, and analysed independently of the sponsor.

Informed consent was obtained from each patient prior to randomisation in the LIPID study, and also prior to samples being taken for biomarker analyses. The study protocol conforms to the Ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by Institutional Human Research Committees.

Statistical Methods

Continuous variables are described as means (with standard deviations) or medians (with 25th and 75th percentiles). As pre-specified in the biomarker analysis plan, MR-proADM was analysed in quartiles: ≤0.381, 0.381 to ≤0.474, 0.474 to ≤0.578 and >0.578 nmol/L. Change in MR-proADM after one year was also categorised in quartiles: ≤-0.0665, -0.06665 to ≤-0.0029, -0.0029 to ≤0.0565 and >0.0565 nmol/L. Comparisons over these quartiles were performed as a test of trend, using a generalised linear model for continuous variables and an ordinal or logistic regression for categorical variables. Change in biomarker concentrations were compared between treatment groups using a Wilcoxon signed rank test. The relationship between baseline MR-proADM and other baseline risk factors was assessed using Spearman's rank correlation coefficient.

The association between baseline MR-proADM quartiles and outcome was assessed using the Cox proportional hazards model after adjustment for treatment and 23 baseline risk

factors; gender, prior stroke, diabetes mellitus, current smoking, hypertension, fasting glucose, total cholesterol, apolipoprotein B, apolipoprotein A1, HDL-cholesterol, triglycerides, age, type of qualifying prior acute coronary syndromes, timing of coronary revascularisation, systolic blood pressure, atrial fibrillation, estimated glomerular filtration rate, body mass index, dyspnoea class, angina grade, white blood cell count, peripheral vascular disease and whether the patient was taking aspirin at baseline. Each of these variables had been shown to be an independent predictor of cardiovascular disease events in published LIPID risk models. [18]

The relationship between change in biomarker concentration and CHD events after 12 months was assessed using Cox regression in a landmark model that included baseline risk factors and baseline MR-proADM concentration. Whether MR-proADM improved discrimination between those who did and those who did not develop events was assessed using the C statistic which was calculated from the time-to-event analysis[19] , and also Net Reclassification Improvement (NRI). To assess risk reclassification following the addition of MR-proADM, the categories used were <7.5%, 7.5% to <10%, 10% to <15% and ≥15% risk of all endpoints during follow-up at 5 years. Improvement in classification was defined as upward movement in risk category in patients who experienced an event, or movement to a lower risk category in those who did not experience such an event.

Finally models testing the prognostic value of both baseline MR-proADM and its change over 12 months were further adjusted for concentrations of BNP and its change.

The pre-specified level of significance was 0.05, except in the context of interaction p-values where this was instead 0.01 due to the large number of comparisons.

For the primary endpoint of major CHD events, of which there were 1100, there was 80% power to detect a hazard ratio of at least 1.19.

Analyses were carried out using SAS 9.2 SAS Institute Inc., Cary, NC, USA.

Results

Baseline characteristics stratified by baseline MR-proADM concentrations

Table 1 shows the baseline demographics, cardiovascular risk factors, and clinical features associated with atherosclerotic cardiovascular disease categorised by MR-proADM quartiles. Individuals with higher MR-proADM concentrations were significantly older and more frequently female (both p<0.001). Further, patients from the higher MR-proADM quartiles more often had recognised cardiovascular risk factors (hypertension, obesity, previous stroke, lower HDL-cholesterol concentrations) and impaired renal function (lower estimated glomerular filtration rate), had a higher estimated global risk score and were less likely to have undergone coronary revascularisation (all p<0.001). Those with the highest MR-proADM concentrations were also more likely to be treated at baseline with antihypertensive medication (angiotensin converting enzyme (ACE) inhibitors, beta-blockers, calcium antagonists) (all p<0.001) and less likely to be treated with aspirin (p<0.01).

MR-proADM showed a moderate correlation with BNP (r=0.54), and a modest correlation with age at randomisation (r=0.36), serum creatinine (r=0.30), and estimated glomerular filtration rate (eGFR) (r=-0.40) (all p<0.001) (Table 2).

Association between baseline MR-proADM concentration and outcomes

Increasing baseline MR-proADM quartiles were strongly associated with the subsequent risk of the composite endpoint of major CHD events (CHD death and non-fatal myocardial infarction) (highest quartile compared to first quartile (HR 1.52, 95% CI 1.26–1.84; p<0.001) and also CHD death (HR 2.21, 95% CI 1.67-2.92; p<0.001), heart failure (HR 2.30, 95% CI 1.78-2.97; p<0.001), and all-cause mortality (HR 1.82, 95% CI 1.49-2.23; p<0.001). There was no association with non-fatal myocardial infarction alone (HR 1.11, 95% CI 0.86-1.42, p=0.43) (Table 3).

Table 3 shows that after additional adjustment for BNP concentration, the associations of baseline MR-proADM concentration appeared slightly attenuated but still remained significant for major CHD events, CHD death, heart failure and all-cause mortality.

Associations with change in MR-proADM concentrations from baseline to one year

An increase in MR-proADM concentration in the first year >0.0565 nmol/l (the highest quartile of change) was associated with a higher risk of major CHD events compared to those individuals with the greatest reduction in concentration of MR-proADM ≤-0.0665 nmol/l (lowest quartile) (HR 1.34, 95%CI 1.08-1.66; p=0.007). This was primarily due to the increased risk of non-fatal myocardial infarction (HR 1.50, 95 % CI 1.12-2.03; p=0.007). Strong associations of change in MR-proADM were also observed for incident heart failure (HR 1.78, 95% CI 1.37-2.30, p<0.001) and all-cause mortality (HR 1.42, 1.15-1.76, p=0.001) (Table 4).

As shown in Table 4, after additional adjustment for baseline and change in BNP concentration, the association of outcome with change of MR-proADM concentration after one year remained significant for incident heart failure and all-cause mortality.

Metrics of discrimination and reclassification for baseline MR-proADM and outcomes

Adding baseline MR-proADM concentration to the set of baseline variables improved discrimination and reclassification for CHD death (C statistic 0.735/0.747; NRI 4.61%, p=0.07) and heart failure (C statistic 0.740/0.751; NRI 4.70%, p=0.05) but not for major CHD events (C statistic 0.665/0.669; NRI 2.36%, p=0.15), non-fatal myocardial infarction (C statistic 0.629/0.629; NRI -0.60%, p=0.62) or all-cause mortality (C statistic 0.702/0.712; NRI 3.69, p=0.06) (Table 5).

Metrics of discrimination and reclassification for change in MR-proADM and outcomes

In a landmark model the change of MR-proADM at one year (additionally adjusted for baseline MR-proADM concentration) strongly improved discrimination and risk reclassification for subsequent heart failure (C statistic 0.737/0.745; NRI 5.60%, p=0.01). For subsequent major CHD events, only moderate risk reclassification was observed (C statistic 0.655/0.659; NRI 3.48%, p=0.02), whereas no significant reclassification was observed for the individual endpoints of non-fatal myocardial infarction (C statistic 0.629/0.633; NRI 1.37%, p=0.43), CHD death (C statistic 0.739/0.742; NRI -1.28%, p=0.50) or all-cause mortality (C statistic 0.706/0.712; NRI 1.29, p=0.50) (Table 5).

Interaction with treatment effect of pravastatin

Table 6 compares the associations of baseline MR-proADM quartiles with study outcomes in both treatment groups. The relative treatment effect of pravastatin was comparable across each baseline quartile of MR-proADM concentrations for each outcome measure, with no evidence of any significant interaction. This constant relative effect translated into an increasing absolute benefit of pravastatin with increasing quartile of MR-proADM, i.e. number needed to treat (NNT) over 5 years decreased for all endpoints: major CHD events (NNT: lowest quartile versus highest quartile 50 vs. 26), CHD death (NNT: 97 vs. 38), non-fatal myocardial infarction (NNT: 84 vs. 54), heart failure (NNT: 184 vs. 65), and all-cause mortality (NNT: 58 vs. 25).

Sex-specific differences in baseline MR-proADM concentrations and change in MR-proADM concentrations during pravastatin treatment

Median baseline MR-proADM concentrations were higher in females (placebo: 0.53 nmol/L (IQR 0.42,0.65 nmol/L) pravastatin: 0.51 nmol/L (IQR 0.40,0.64 nmol/L) than in males (placebo:0.46 nmol/L (IQR 0.38,0.57 nmol/L), pravastatin: 0.47 nmol/L (IQR 0.36,0.56nmol/L)) (Table 7). After one year of treatment, MR-proADM concentration decreased by a small but significantly greater extent in those patients taking pravastatin

(p=0.03).There were no differences between genders in the magnitude of this change in MR-proADM with pravastatin.

Discussion

The present data from the large, randomised, placebo-controlled LIPID study focussed on the predictive value of baseline MR-proADM concentration and the change in its concentration after one year in patients with stable CHD. In this prespecified biomarker analysis we showed an association between baseline MR-proADM concentrations and the incidence of subsequent major clinical events. Higher baseline MR-proADM concentrations were an independent predictor of future major CHD events, particularly CHD death, and also predicted heart failure and all-cause mortality. In addition, patients who had greater reduction in MR-proADM concentrations after one year, had a lower risk of CHD events, primarily due to a lower risk for non-fatal myocardial infarction, and also a lower risk of subsequent heart failure and all-cause mortality. Despite the correlation of MR-proADM with BNP concentration, the associations of baseline MR-proADM concentration persisted after additional significant adjustment for baseline BNP. Interestingly, the change in MR-proADM after one year was still highly significant for subsequent heart failure hospitalisation or death after further adjusting for BNP concentration.

The findings related to the effect of change in MR-proADM on subsequent events are particularly noteworthy. This is a very important and robust test of the relevance of a biomarker, but is rarely assessed.

MR-proADM baseline concentrations added borderline significant discriminative effect beyond classical risk factors for CHD death and heart failure, but not for major CHD events or myocardial infarction. The absolute changes in C statistic were relatively small. The greatest risk reclassification was found for heart failure by adding the change of MR-proADM concentration after one year (NRI 5.60%). There were no significant differences in the relative treatment effect of pravastatin according to baseline MR-proADM quartiles.

Association of baseline MR-proADM with laboratory parameters and classical risk factors

Our finding that increased MR-proADM concentrations were associated with impaired renal function is consistent with previous studies.[20] MR-proADM was also associated with predictors of CHD mortality, including age, clinical evidence of severe cardiac and cerebrovascular disease (previous stroke, higher global risk score), hypertension, obesity, diabetes and BNP concentration.[13,16, 18, 21] The correlation with BNP concentration is consistent with the findings of others.[22]

Associations of baseline MR-proADM concentrations and change of MR-proADM with cardiovascular outcomes

Results in earlier trials concerning the predictive value of adrenomedullin were ambiguous. However, after the establishment of modern assays to detect stable precursors, MR-proADM has evolved into an important biomarker in cardiac risk prediction. [23, 24]

Khan et al. assessed the prognostic value of MR-proADM in patients with acute myocardial infarction in the Leicester Acute Myocardial Infarction Peptide (LAMP) studies in ST-Elevation Myocardial Infarction [15] and non–ST-elevation Myocardial Infarction[25]. Patients with higher concentrations of MR-proADM were at increased risk of death and incident heart failure. In the LAMP studies, blood samples were drawn as early as 3 to 5 days after the qualifying event. In contrast in our present study, baseline blood samples were drawn at a median of 13.9 months (IQR 7.9, 25.0) after the qualifying event. Therefore MR-proADM concentrations in the LAMP studies may reflect the early pathophysiological and remodelling processes after myocardial infarction rather than testing their value as a prognostic marker in long-term follow-up in patients with stable CHD.

The present data showing that baseline MR-proADM concentrations predicted CHD death and heart failure, but not non-fatal myocardial infarction are in accordance with the findings of the Athero*Gene* Study which investigated MR-proADM concentrations in a smaller number

of individuals (n=2,240) with stable angina or acute coronary syndrome and evaluated their prognostic impact on cardiovascular events during a follow-up period of almost 4 years.[16] In Athero*Gene*, MR-proADM concentrations were independently associated with fatal or non-fatal cardiovascular events.

Sabatine et al. [22] showed that in patients with stable CHD and preserved ejection fraction, biomarkers reflecting cardiovascular stress (e.g. MR-proADM, natriuretic peptides) were more predictive of CHD death and incident heart failure, than the composite of CHD death and myocardial infarction. This is similar to our findings.

In our study the association of MR-proADM concentrations with subsequent heart failure events was even stronger than for the primary endpoint of major CHD events. Since reduction in left ventricular function is a common result of the structural changes in the heart caused by CHD, our observations may be explained by the different pathophysiological processes involved. In animal models, MR-proADM is increased in response to pressure-and volume-overload and protects against fibrosis and hypertrophy.[26-28] This suggests a potential counter-regulatory effect protecting against structural cardiac changes. This is supported by our finding that patients who had the greatest reduction in MR-proADM concentration after one year compared to those with little change or increasing MR-proADM concentrations showed a reduced risk of major CHD events, non-fatal myocardial infarction, heart failure and all-cause mortality.

Of particular note, in the PEACE study[22] trandolapril was most effective in those individuals in the upper quartile of MR-proADM concentrations. This biomarker/therapy interaction was not observed for N-terminal pro-B-type natriuretic peptide. Our findings are also congruent with those in the HOPE biomarker study.[29]

<u>What are the implications of these data?</u>

Together with our observation that changes in MR-proADM concentrations can impact on incident heart failure and other major events even after adjusting for BNP concentration, MR-

proADM may have the potential to inform therapeutic decision-making in stable CHD patients. Single baseline determination of MR-proADM, because it predicts death and heart failure as well as recurrent major CHD events, and serial assessment of the change of MR-proADM potentially has important clinical utility. Furthermore, MR-proADM concentrations predict heart failure beyond clinical variables such as hypertension and body mass index, and additionally BNP concentration, and might guide the modification or intensity of therapy aimed at preventing or managing heart failure. However, this possibility of MR-proADM guided therapy needs to be tested prospectively

Effect of pravastatin therapy

After one year of treatment with pravastatin, MR-proADM concentration decreased to a small but significantly greater extent in those patients taking pravastatin. It is possible but remains speculative whether this could partly contribute to the reduced risk for subsequent events in patients receiving statins.

The relative treatment effect of pravastatin on outcomes was very similar across each baseline quartile of MR-proADM. However, with increasing MR-proADM quartiles, the NNT decreased for all endpoints. Thus, patients in the higher MR-proADM quartiles experienced a greater absolute benefit because of their higher risk. This finding suggests that such patients need the most intensive surveillance with regard to modification of cardiovascular risk factors and compliance with secondary preventive strategies. Recognising and treating aggressively on the basis of these findings may result in major benefits for individuals with highly elevated MR-proADM concentrations.

Study limitations

The LIPID study was conducted some years ago. However the cohort has ongoing major relevance to current clinical management since it provides a unique dataset with long-term follow-up of a large group of typical CHD patients who had previously been admitted to

hospital with myocardial infarction or unstable angina. Furthermore patients had a broad range of cholesterol levels reflecting those in usual clinical practice. Importantly there were relatively few exclusion criteria and of just over 11,000 screened patients, 9,014 were subsequently randomised to receive pravastatin or placebo. Biomarker levels were available in a high proportion, 7,863 patients at baseline and 6,658 after one year. Background therapy was also quite similar to therapies which are currently recommended, although usage of angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers was less than in contemporary practice. Also use of beta-blockers and ACE inhibitors was by chance, higher in those with the highest MR-proADM levels. Since commencement of the LIPID trial the most significant changes in management of coronary heart disease patients have been dual antiplatelet therapy and the more frequent use of primary angioplasty in acute myocardial infarction. However these advances are less relevant to stable CHD patients, as were recruited to the LIPID study. However it is acknowledged that preservation of left ventricular function could modulate the impact of the biomarkers investigated. In addition, use of implantable cardioverter defibrillators have improved survival of selected CHD patients, although those known to have a very low ejection fraction were excluded from the LIPID study. Although diagnostic criteria for myocardial infarction have evolved, the LIPID cohort still represents stable CHD patients randomised at a median of 1 year after their qualifying event.

The models used to assess the effects of change in MR-proADM were restricted to events after the 12 month sample was taken. Therefore, these data provide no information on very high risk patients, who had an event which may have been fatal, within the first 12 months after randomisation. Also the LIPID study included predominantly males (83%). This is of particular interest since females had higher MR-proADM concentrations. Gender-specific analyses would be of interest in future studies.

Finally, the diagnosis of heart failure at baseline was based on clinical assessment rather than echocardiographic or angiographic parameters. However, incident heart failure during

follow-up was diagnosed on the basis of hard end-points, either need for hospitalisation or death due to heart failure.

Despite these limitations, we believe that the observed associations of both baseline MR-proADM and its change after one year with major clinical outcomes, which importantly persisted after adjustment for BNP concentration, have major relevance for contemporary patient management and research.

Conclusions

In conclusion, determining MR-proADM baseline concentration in stable CHD patients with previous acute coronary syndromes may be a valuable tool for physicians to identify those at particular need for intensified surveillance of risk factors and for potential complications of CHD. This could also inform more intensive measures to improve compliance with statins and other evidence-based therapies. Evaluating the change of MR-proADM concentration during monitoring of patients may also help to identify patients at particular risk of incident heart failure beyond use of brain natriuretic peptides for this purpose.

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Table 1: Baseline characteristics stratified by baseline MR-proADM* concentrations

	MR-proADM ≤0.381 pg/mL	MR-proADM 0.381-0.474 pg/mL	MR-proADM 0.474-0.578 pg/mL	MR-proADM >0.578 pg/mL	P values (trend)
N	1961	1971	1969	1962	
MR-proADM (nmol/L)	0.3 (0.1)	0.4 (0.0)	0.5 (0.0)	0.7 (0.1)	
Age at randomisation	58 (51 - 64)	60 (53 - 65)	63.0 (58 - 68)	67 (62 - 70)	<0.001
Female	262 (13%)	262 (13%)	321 (16%)	488 (25%)	<0.001
Baseline characteristics					
Months from qualifying event	14.0 (7.9-25.2)	14.3 (7.9-24.7)	13.5 (7.9-24.5)	14.1 (8.0-25.5)	0.80
Current smoker	180 (9%)	175 (9%)	182 (9%)	198 (10%)	0.28
Hypertension	759 (39%)	716 (36%)	801 (41%)	1015 (52%)	<0.001
Diabetes mellitus	157 (8%)	137 (7%)	165 (8%)	217 (11%)	<0.001
Obesity	315 (16%)	299 (15%)	318 (16%)	465 (24%)	<0.001
Previous stroke	54 (3%)	70 (4%)	70 (4%)	128 (7%)	<0.001
Systolic blood pressure (mmHg)	131 (18)	132 (19)	135 (19)	138 (20)	<0.001

	MR-proADM ≤0.381 pg/mL	MR-proADM 0.381-0.474 pg/mL	MR-proADM 0.474-0.578 pg/mL	MR-proADM >0.578 pg/mL	P values (trend)
Diastolic blood pressure (mmHg)	80 (11)	80 (11)	81 (11)	81 (11)	0.24
Baseline lipids					
Total cholesterol (mmol/L)	5.7 (0.8)	5.6 (0.8)	5.7 (0.8)	5.7 (0.8)	0.44
LDL-cholesterol (mmol/L)	3.9 (0.7)	3.9 (0.7)	3.9 (0.7)	3.9 (0.8)	0.90
HDL-cholesterol (mmol/L)	1.0 (0.2)	1.0 (0.2)	0.9 (0.2)	0.9 (0.2)	<0.001
Triglycerides (mmol/L)	1.5 (1.1 - 2.2)	1.5 (1.1 - 2.1)	1.6 (1.2 - 2.1)	1.7 (1.2 - 2.3)	<0.001
Total to HDL-cholesterol ratio	6.1 (1.6)	6.2 (1.5)	6.2 (1.5)	6.3 (1.6)	<0.001
Previous coronary revascularisation					
No revascularisation	1075 (55%)	1160 (59%)	1138 (58%)	1237 (63%)	<0.001
Percutaneous coronary intervention only	287 (15%)	220 (11%)	208 (11%)	155 (8%)	
Coronary artery bypass graft only	526 (27%)	514 (26%)	570 (29%)	531 (27%)	

	MR-proADM ≤0.381 pg/mL	MR-proADM 0.381-0.474 pg/mL	MR-proADM 0.474-0.578 pg/mL	MR-proADM >0.578 pg/mL	P values (trend)
Percutaneous coronary intervention and coronary artery bypass graft	73 (4%)	77 (4%)	53 (3%)	39 (2%)	
Qualifying event					
No myocardial infarction	704 (36%)	685 (35%)	736 (37%)	718 (37%)	0.17
Single myocardial infarction	1072 (55%)	1082 (55%)	1003 (51%)	958 (49%)	
Multiple myocardial infarctions	185 (9%)	204 (10%)	230 (12%)	286 (15%)	
Medications					
Aspirin	1632 (83%)	1672 (85%)	1631 (83%)	1566 (80%)	<0.01
ACE inhibitors	241 (12%)	224 (11%)	305 (15%)	484 (25%)	<0.001
Beta-blocker	827 (42%)	932 (47%)	949 (48%)	983 (50%)	<0.001
Calcium antagonist	609 (31%)	602 (31%)	694 (35%)	783 (40%)	<0.001
Global risk score [8]					
Low risk	804 (41%)	759 (39%)	665 (34%)	482 (25%)	<0.001

	MR-proADM ≤0.381 pg/mL	MR-proADM 0.381-0.474 pg/mL	MR-proADM 0.474-0.578 pg/mL	MR-proADM >0.578 pg/mL	P values (trend)
Medium risk	570 (29%)	536 (27%)	477 (24%)	434 (22%)	
High risk	398 (20%)	467 (24%)	553 (28%)	628 (32%)	
Very high risk	189 (10%)	209 (11%)	274 (14%)	418 (21%)	
Mean risk score	5.1 (3.3)	5.4 (3.3)	5.9 (3.5)	6.9 (3.5)	<0.001
Baseline biomarker concentrations					
eGFR (mL/min/1.73 m²)	77 (66 - 87)	74 (66 - 83)	69 (61 - 78)	60 (51 - 69)	<0.001
White blood cell count (cells/µl)	6.9 (5.9 - 8.1)	6.9 (5.8 - 8.1)	7.1 (6.0 - 8.2)	7.3 (6.2 - 8.5)	<0.001
BNP (pg/mL)	8.4 (2.0 - 19.1)	20.3 (10.3 - 37.8)	29.6 (15.2 - 54.0)	49.4 (24.8 - 95.4)	<0.001

*MR-proADM = midregional proadrenomedullin

Statistics presented are mean (standard deviation), median (Q1 – Q3) or N (%)
P-values for trend for continuous variables are from a generalised linear model, and for categorical variables from an ordinal or logistic regression.

Table 2: Baseline correlations with MR-proADM concentration

	MR-proADM
BNP concentration	0.54
Troponin I concentration	0.12
Age	0.36
Myocardial infarction	0.09
Systolic blood pressure	0.14
Diastolic blood pressure	0.01
Global Risk score [18]	0.19
White blood cell count	0.08
Serum creatinine	0.30
Estimated glomerular filtration rate	-0.40
Total cholesterol	0.01

The relationship between baseline MR-proADM concentration and other baseline risk factors was assessed using Spearman's rank correlation coefficient. All baseline correlations were highly significant ($p<0.001$), except for diastolic blood pressure ($p=0.18$) and total cholesterol ($p=0.40$)

Table 3: Cox regression models for various endpoints with baseline MR-proADM quartiles

		Event rates		Adjusted for other variables only^		Adjusted for **BNP** and other variables^	
Endpoint	MR-proADM	Events/Total	5 yr event rate	HR (95% CI)	p value**	HR (95% CI)	p value**
Major CHD events	<= 0.381	204/1961	8.5	1	<.001	1	0.03
	0.381-0.474	220/1971	9.2	1.05 (0.87, 1.27)		0.99 (0.81, 1.21)	
	0.474-0.578	280/1969	11.8	1.26 (1.05, 1.52)		1.14 (0.93, 1.39)	
	> 0.578	396/1962	17.4	1.52 (1.26, 1.84)		1.27 (1.03, 1.57)	
Non-fatal myocardial	<= 0.381	136/1961	6	1	0.43	1	0.33
infarction	0.381-0.474	150/1971	6.3	1.09 (0.86, 1.38)		1.12 (0.88, 1.43)	
	0.474-0.578	157/1969	7.1	1.11 (0.88, 1.41)		1.16 (0.90, 1.49)	
	> 0.578	176/1962	8	1.11 (0.86, 1.42)		1.15 (0.87, 1.52)	
CHD death	<= 0.381	79/1961	3.2	1	<.001	1	0.03
	0.381-0.474	82/1971	3.4	0.97 (0.71, 1.33)		0.80 (0.58, 1.10)	
	0.474-0.578	152/1969	6	1.64 (1.24, 2.18)		1.22 (0.90, 1.64)	
	> 0.578	252/1962	10.9	2.21 (1.67, 2.92)		1.41 (1.04, 1.93)	
Heart Failure	<= 0.381	92/1961	4.3	1	<.001	1	0.004

		Event rates		Adjusted for other variables only^		Adjusted for **BNP** and other variables^	
Endpoint	MR-proADM	Events/Total	5 yr event rate	HR (95% CI)	p value**	HR (95% CI)	p value**
	0.381-0.474	111/1971	4.8	1.24 (0.94, 1.65)		1.05 (0.78, 1.40)	
	0.474-0.578	161/1969	6.7	1.50 (1.15, 1.96)		1.15 (0.86, 1.52)	
	> 0.578	326/1961	14.9	2.30 (1.78, 2.97)		1.52 (1.15, 2.03)	
All-cause mortality	<=0.381	157/1961	6.2	1	<.001	1	0.03
	0.381-0.474	148/1971	5.9	0.88 (0.70, 1.10)		0.76 (0.60, 0.96)	
	0.474-0.578	251/1969	9.3	1.31 (1.07, 1.61)		1.04 (0.84, 1.30)	
	>0.578	417/1962	17.2	1.82 (1.49, 2.23)		1.28 (1.02, 1.61)	

^HR and 95% CI are adjusted for baseline variables: Gender, treatment, stroke, diabetes, smoking, hypertension, total cholesterol, apo B, apo A1, HDL-c, age, Nature of prior ACS, timing of coronary revascularisation, systolic blood pressure, atrial fibrillation, eGFR, BMI, dyspnoea class, angina grade, white cell count, peripheral vascular disease, aspirin use at baseline.
Adjustment for BNP included Baseline BNP quartiles.

Table 4: Landmark models with outcomes associated with quartiles of change in MR-proADM

		Adjusted for baseline variables only^		Adjusted for baseline **BNP, change in BNP** and other baseline variables^	
Outcome	Change in MR-proADM (nmol/L)	HR (95% CI)	p value	HR (95% CI)	p value
Major CHD events	<= -0.0665	1	0.007	1	0.08
	-0.0665 - -0.0029	1.10 (0.89, 1.36)		1.07 (0.86, 1.33)	
	-0.0029 - 0.0565	1.12 (0.90, 1.39)		1.06 (0.85, 1.34)	
	> 0.0565	1.34 (1.08, 1.66)		1.23 (0.97, 1.55)	
Non-fatal myocardial infarction	<= -0.0665	1	0.007	1	0.06
	-0.0665 - -0.0029	1.37 (1.03, 1.83)		1.29 (0.96, 1.72)	
	-0.0029 - 0.0565	1.26 (0.93, 1.70)		1.15 (0.84, 1.56)	
	> 0.0565	1.50 (1.12, 2.03)		1.35 (0.98, 1.85)	
CHD death	<= -0.0665	1	0.14	1	0.55

		Adjusted for baseline variables only^		Adjusted for baseline **BNP, change in BNP** and other baseline variables^	
Outcome	Change in MR-proADM (nmol/L)	HR (95% CI)	p value	HR (95% CI)	p value
	-0.0665 - -0.0029	0.85 (0.64, 1.14)		0.84 (0.62, 1.14)	
	-0.0029 - 0.0565	0.95 (0.71, 1.27)		0.96 (0.70, 1.30)	
	> 0.0565	1.24 (0.93, 1.65)		1.10 (0.81, 1.50)	
Heart Failure	<= -0.0665	1	<.001	1	0.002
	-0.0665 - -0.0029	1.13 (0.86, 1.48)		1.08 (0.82, 1.43)	
	-0.0029 - 0.0565	1.22 (0.92, 1.61)		1.12 (0.84, 1.50)	
	> 0.0565	1.78 (1.37, 2.30)		1.57 (1.19, 2.07)	
All-cause mortality	<= -0.0665	1	0.001	1	0.02
	-0.0665 - -0.0029	0.92 (0.74, 1.15)		0.89 (0.71, 1.12)	
	-0.0029 - 0.0565	1.09 (0.88, 1.36)		1.05 (0.84, 1.32)	
	> 0.0565	1.42 (1.15, 1.76)		1.31 (1.04, 1.64)	

^ HR and 95% CI are adjusted for baseline variables: Gender, treatment, stroke, diabetes, smoking, hypertension, total cholesterol, apo B, apo A1, HDL-c, age, Nature of prior ACS, timing of coronary revascularisation, systolic blood pressure, atrial fibrillation, eGFR, BMI, dyspnoea class, angina grade, white cell count, peripheral vascular disease, aspirin use at baseline, and also baseline Mr-proADM concentration.
Adjustment for BNP included Baseline BNP quartiles and quartiles of change in BNP.

Table 5: Discrimination and reclassification after adding MR-proADM concentrations to models at baseline and considering change in MR-proADM after 1 year

		NRI		C statistic	
	Outcome	NRI (%)	p-value	Without MR-proADM	With MR-proADM
MR-proADM baseline	Major CHD events	2.36	0.15	0.665	0.669
	• Non-fatal myocardial infarction	-0.60	0.62	0.629	0.629
	• CHD death	4.61	0.07	0.735	0.747
	Heart failure	4.39	0.07	0.740	0.751
	All-cause mortality	3.69	0.06	0.702	0.712
MR-proADM change	Major CHD events	3.48	0.02	0.655	0.659
	• Non-fatal myocardial infarction	1.37	0.43	0.629	0.633
	• CHD death	-1.28	0.50	0.739	0.742
	Heart failure	5.60	0.01	0.737	0.745
	All-cause mortality	1.29	0.50	0.706	0.710

Table 6: Effect of pravastatin on study outcomes in subjects in the different MR-proADM quartiles

Outcome	MR-proADM quartile (nmol/L)	Placebo	Pravastatin	NNT	HR (95% CI)	p- trend
		N (% 5-year events)	N (% 5-year events)			
Major CHD events	≤ 0.381	114 (9.5)	90 (7.6)	50	0.74 (0.56, 0.98)	0.94
	0.381-0.474	123 (10.0)	97 (8.3)	48	0.83 (0.64, 1.08)	
	0.474-0.578	151 (13.0)	129 (10.6)	37	0.80 (0.63, 1.01)	
	> 0.578	222 (19.1)	174 (15.8)	26	0.76 (0.62, 0.93)	
• Non-fatal myocardial infarction	≤ 0.381	71 (6.0)	65 (6.0)	84	0.86 (0.61, 1.20)	0.41
	0.381-0.474	84 (7.0)	66 (5.5)	72	0.83 (0.60, 1.14)	
	0.474-0.578	85 (7.8)	72 (6.5)	65	0.79 (0.58, 1.09)	
	> 0.578	101 (9.5)	75 (6.6)	54	0.72 (0.54, 0.97)	
• CHD death	≤ 0.381	50 (4.4)	29 (2.0)	97	0.55 (0.35, 0.86)	0.4
	0.381-0.474	46 (3.6)	36 (3.3)	118	0.84 (0.54, 1.29)	
	0.474-0.578	80 (6.4)	72 (5.6)	68	0.85 (0.61, 1.16)	
	> 0.578	141 (11.6)	111 (10.2)	38	0.77 (0.60, 0.98)	

Outcome	MR-proADM quartile (nmol/L)	Placebo	Pravastatin	NNT	HR (95% CI)	p- trend
		N (% 5-year events)	N (% 5-year events)			
Heart failure	≤ 0.381	53 (5.1)	39 (3.5)	184	0.68 (0.45, 1.03)	0.41
	0.381-0.474	55 (4.8)	56 (4.8)	195	1.09 (0.75, 1.58)	
	0.474-0.578	87 (7.6)	74 (5.8)	125	0.80 (0.59, 1.09)	
	> 0.578	166 (15.3)	161 (14.6)	65	0.94 (0.76, 1.17)	
All-cause mortality	≤ 0.381	88 (7.4)	69 (5.1)	58	0.74 (0.54, 1.02)	0.76
	0.381-0.474	87 (6.5)	61 (5.4)	66	0.75 (0.54, 1.04)	
	0.474-0.578	140 (10.3)	111 (8.3)	42	0.74 (0.58, 0.95)	
	> 0.578	232 (17.9)	185 (16.4)	25	0.78 (0.64, 0.94)	

Table 7: Baseline, Year 1 and change in MR-proADM concentration by sex and randomised treatment

	Placebo		Pravastatin	
	Females	Males	Females	Males
Baseline (nmol/L)	0.53 (0.42, 0.65)	0.46 (0.38, 0.57)	0.51 (0.40, 0.64)	0.47 (0.38, 0.56)
Year 1 (nmol/L)	0.51 (0.40, 0.63)	0.46 (0.37, 0.57)	0.50 (0.40, 0.63)	0.46 (0.36, 0.56)
Change (nmol/L)	-0.00 (-0.08, 0.06)	0.00 (-0.06, 0.06)	-0.02 (-0.09, 0.06)	-0.00 (-0.07, 0.05)

Data presented are median (IQR).