Multiple cardiac biomarkers to improve prediction of cardiovascular events

ORIGINAL ARTICLE

Multiple cardiac biomarkers to improve prediction of cardiovascular events: Findings from the Generation Scotland Scottish Family Health Study

Running title: Combined biomarker approach & CVD

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Nonstandard abbreviations

NT-proBNP	N-terminal pro-B-type natriuretic peptide
GDF-15	growth differentiation factor-15
cTnI	cardiac troponin I
cTnT	cardiac troponin T
CRP	C-reactive protein
MACE	major adverse cardiovascular events
GS:SFHS	Generation Scotland Scottish Family Health study
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
LoD	limit of detection
LoB	limit of blank
UKNEQAS	National External Quality Assurance Scheme
ICD-10	10^{th} revision of the International Classification of Diseases
IDI	integrated discrimination index
NRI	net reclassification index

Abstract

Background: Many studies have investigated whether single cardiac biomarkers improve cardiovascular risk prediction for primary prevention but whether a combined approach could further improve risk prediction is unclear. We aimed to test a sex-specific combined cardiac biomarker approach for cardiovascular risk prediction.

Methods: In the Generation Scotland Scottish Family Health Study, N-terminal pro-B-type natriuretic peptide (NT-proBNP), growth differentiation factor-15 (GDF-15), cardiac troponin I (cTnI), cardiac troponin T (cTnT), and C-reactive protein (CRP) were measured in stored serum using automated immunoassays. Sex-specific Cox models that included SCORE2 risk factors evaluated addition of single and combined biomarkers for prediction of major adverse cardiovascular events (MACE). Combined biomarker models were compared to a baseline model that included SCORE2 factors.

Results: The study population comprised 18,383 individuals (58.9% women, median age of 48 years [25^{th}-75^{th} percentile, 35-58 years]). During the median follow up of 11.6 (25^{th}-75^{th} percentile, 10.8-13.0) years, MACE occurred in 942 (5.1%) individuals. The greatest increase in discrimination with addition of individual biomarkers to base model was for women GDF-15 and for men NT-proBNP (change in c-index: +0.010 for women and +0.005 for men). For women, combined biomarker models that included GDF-15 and NT-proBNP (+0.012) or GDF-15 and cTnI (+0.013), but not CRP or cTnT, further improved discrimination. For men, combined biomarker models that included NT-proBNP and GDF-15 (+0.007), NT-proBNP and cTnI (+0.006), or NT-proBNP and CRP (+0.008), but not cTnT, further improved discrimination.

Conclusions: A combined biomarker approach, particularly the use of GDF-15, NT-proBNP and cTnI, further refined cardiovascular risk estimates.

Keywords: Biomarkers, cardiovascular, risk factors, general population

Introduction

Cardiovascular risk estimation is one of the cornerstones of the primary prevention of cardiovascular disease (1, 2). A biomarker-driven approach may refine cardiovascular risk estimates because disease-specific biomarkers can provide additional information about the presence and extent of asymptomatic cardiovascular disease which could help improve individual risk prediction.

Previous studies have shown that N-terminal pro-B-type natriuretic peptide (NT-proBNP), growth differentiation factor-15 (GDF-15), cardiac troponin I (cTnI), cardiac troponin T (cTnT), and C-reactive protein (CRP) predict cardiovascular events in people with established cardiovascular disease, and in the general population (3-12). Elevations in these biomarkers reflect different underlying pathophysiological features of cardiovascular disease, including myocardial ischemia or injury (cTnI, cTnT), cardiac wall stretch or remodeling (NT-proBNP), inflammation (CRP) and generalized tissue damage (GDF-15). Previous studies have investigated whether single or combined cardiac biomarker approaches may improve risk prediction for primary prevention of cardiovascular disease (13-16). However, whether a combined biomarker approach, using assays relevant to contemporary clinical biochemistry settings, could further improve prediction of risk in both sexes is unclear. Important sex differences are observed between the relationship of cardiac biomarkers and cardiovascular disease (17-19), and studies of large size are required for sex-specific evaluation of candidate cardiac biomarkers and their combinations.

We hypothesized that cardiac biomarkers would enhance the prediction of cardiovascular events compared to using conventional risk factors alone, and that an approach that used a

combination of biomarkers would be superior to the use of any single biomarker in both women and men. Accordingly, we evaluated associations between NT-proBNP, GDF-15, cTnI, cTnT and CRP and major adverse cardiovascular events (MACE) in the Generation Scotland Scottish Family Health Study (GS:SFHS).

Methods

Because of the sensitive nature of the data collected for this study, requests to access the dataset from qualified researchers trained in human subject confidentiality protocols should be sent to the Generation Scotland management team at access@generationscotland.org.

Study population

The GS:SFHS is a family-based cohort that enrolled 24,090 participants aged between 18 and 98 years (20, 21). Briefly, individuals between 35 and 65 years old were identified at random from participating general practices in Scotland between February 2006 and March 2011. Participants were then asked to identify one or more first-degree relatives ≥18 years old who would also be able to participate. For this study, we excluded participants with cardiovascular disease at baseline, those who had missing cardiac biomarker measurements, or who did not attend the clinical survey. As GDF-15 concentrations are temporarily substantially increased during pregnancy, we also excluded pregnant women (self-reported or when GDF-15 concentrations were >10,000 pg/mL in women aged ≤45 years) (22). Participants completed a health questionnaire, and clinical characteristics were measured using a standardized protocol. The cohort is almost enitrely of White ethnicity (99%) (20) and therefore is not further reported in this study. Study participants provided written informed consent, including linkage to their medical records. Ethical approval for the GS:SFHS study was obtained from the National Health Service Tayside Research Ethics Committee (Research Ethics Committee reference

number 05/S1401/89). The study was conducted according to principles of the Declaration of Helsinki and follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Biomarker measurements

Serum concentrations of NT-proBNP, GDF-15, high-sensitivity cTnT and high-sensitivity CRP were measured on a Cobas e411 analyser (Roche Diagnostics, Basal, Switzerland). Serum concentration of high-sensitivity cTnI was measured on an ARCHITECT i1000SR analyser (Abbott Diagnostics, Chicago, IL, USA). cTnI and cTnT were measured on a first thaw (measured 2016-2017) (3), with NT-proBNP and GDF-15 measured on a second thaw (measured 2020-2021), and CRP on a third thaw (measured 2021-2022). For NT-proBNP, GDF-15 and CRP, the limit of detection (LoD) is set to 10 pg/mL, 400 pg/mL, and 0.1 mg/L by the manufacturer, respectively. For these biomarkers we reported anything less than the LoD at LoD/2 for continuous analysis (5 pg/mL for NT-proBNP, 200 pg/mL for GDF-15, 0.05 mg/L for CRP). For cTnT, the limit of blank (LoB) and LoD are 3 ng/L and 5 ng/L according to the manufacturer, respectively. For cTnI, the LoB and LoD are 1.2 ng/L and 1.9 ng/L, respectively (23). For the primary analysis cTnT and cTnI concentrations below the LoB are set to the LoB value divided by 2 (cTnT, 1.5 ng/L; cTnI, 0.6 ng/L). Proportions of samples above the LoB or LoD are reported in the Supplemental Data (***Supplemental Table 1***). During the conduct of this study, we participated in the National External Quality Assurance Scheme (UKNEQAS) for selected biomarkers (cTnI, cTnT, and NT-proBNP). The assays were calibrated, and quality controlled using the manufacturers' reagents.

Clinical outcomes

We used the Information Services Division NHS record linkage for Scotland to collect non-fatal cardiovascular events and cause-specific deaths data until the end of August 2021. Information on cause of death was obtained using the NHS Central Register. Non-fatal cardiovascular events and cause-specific deaths were classified using the 10th revision of the International Classification of Diseases (ICD-10). The primary outcome was a composite endpoint of MACE that included non-fatal myocardial infarction (I21-I22), non-fatal stroke (I63-I64, G45) or cardiovascular death (I00-I99). Secondary outcomes were the individual end-points of myocardial infarction (non-fatal and fatal, I21-I22), ischemic stroke (non-fatal and fatal, I63-I64, G45), cardiovascular death (I00-I99) and non-cardiovascular death (other ICD-10 codes).

Statistical analysis

The correlation between circulating biomarkers was assessed by Spearman correlation. We determined the proportion of individuals above either diagnostic or prognostic biomarker thresholds according to clinical guidelines or established thresholds for the normal range (NT-proBNP >125 pg/mL (24), GDF-15 >4000 pg/mL (25), cTnI >26.2 ng/L (26), cTnT >14 ng/L (26) and CRP >2 mg/L (27).

We used sex-specific Cox proportional hazard regression models to quantify the relationship between individual biomarkers and MACE. We assessed the impact of using a competing risk framework on biomarker-MACE risk associations, and concluded the differences in risk associations were so marginal that implementing a competing risk framework was not justified in this study. Adjusted sex-specific regression models included the SCORE2 risk factors (age, smoking status, systolic blood pressure, diabetes mellitus, total cholesterol and high-density lipoprotein cholesterol) and as such did not include adjustment for body mass index (28).

Biomarkers were entered in the model as continuous variables. For each biomarker, we applied $\log_2$ transformation and examined them per 1 SD increase in the model accordingly. We bootstrapped the ratio of the hazard ratios (HRs) to compare the strength of the association of individual biomarkers with MACE in the adjusted model, using NT-proBNP as the referent. We constructed HR plots to illustrate the relationship between biomarkers and MACE and used natural cubic splines to account for non-linear relationships between a biomarker and MACE. The proportional hazards assumption was tested by plotting Schoenfeld residuals.

We evaluated combined biomarker approaches in relation to MACE using sex-specific Cox proportional hazard regression models. We assessed all possible combinations for NT-proBNP, GDF-15, cTnI, cTnT, and CRP and entered the biomarkers as continuous variables into the model ($\log_2$ transformed and examined per 1 SD in the model). Similar covariates were included in the models as in the single biomarker models. We also evaluated discrimination for each biomarker individually and in combination using the Harrell c-statistic, the integrated discrimination index (IDI), and net reclassification index (NRI, continuous and categorical). Testing every possible biomarker combination increases the number of statistical tests conducted, but allows each biomarker combination to be evaluated on the basis of incremental discriminative ability. In addition, the age-specific performance of biomarker models was evaluated for those aged <40 years and ≥40 years.

Secondary analyses were conducted to verify the robustness of our findings. First, we set cTnI and cTnT concentrations below the LoD at the LoD divided by 2 (rather than using LoB). Second, we evaluated discrimination of biomarker models compared to a base model using SCORE2 risk factors and socioeconomic deprivation status. Socioeconomic deprivation status was determined using the Scottish Index of Multiple Deprivation 2009 score, which is derived

from participants' postal codes and compiled using 7 domains of deprivation (income, employment, education, health, access to services, crime, and housing) (29). Third, we additionally evaluated the kidney biomarker creatinine in relation to our primary outcome; we used raw creatinine rather than estimated glomerular filtration rate (eGFR) as a biomarker in order to avoid adjusting eGFR for risk factors already included in its calculation (i.e. age). And finally, we evaluated the associations between biomarkers and secondary outcomes. For completion, we also evaluated the association for all-cause death.

Familial clustering did not affect our analyses, and therefore we only present results from analyses without adjustment for clustering. Multiple imputation by chained equations was used to account for missing data for risk factors (but not missing biomarker concentrations) in the Cox regression models (ten imputed data sets). Statistical analysis was performed using R version 3.6.2.

Results

Clinical characteristics of study population

The cohort comprised 18,383 individuals (58.9% women, median age 48 [25^{th}-75^{th} percentile, 35-58; range 18-94] years; ***Table 1***). Cardiac biomarker concentrations were generally low; 15.0% had an NT-proBNP above 125 pg/mL, 0.8% had a GDF-15 >4000 pg/ml, 0.8% had a cTnI >26.2 ng/L, 2.6% had a cTnT >14 ng/L, and 33.9% had a CRP >2 mg/L.

MACE occurred in 717 (4.0%) of individuals over ten years (***Supplemental Table 2***) and in 942 (5.1%) individuals during the total median follow up of 11.6 (25^{th}-75^{th} percentile, 10.8-13.0) years. In both women and men, baseline concentrations of biomarkers were higher in

those who later experienced MACE compared to those who did not (***Table 1, Supplemental Table 3***). We observed moderate and broadly similar correlations between circulating cardiac biomarkers, with CRP generally showing the weakest correlation with other biomarkers (***Supplemental Fig. 1***).

The association of circulating biomarkers with cardiovascular events

In unadjusted single biomarker models, NT-proBNP had numerically the strongest and CRP had the weakest association with MACE in both sexes (***Fig. 1-2, Table 2***). After adjusting for conventional risk factors included in the SCORE2 risk equation, the HR of NT-proBNP per 1 SD increase on the log scale was 1.56 (95%CI 1.38-1.75) and 1.34 (95%CI 1.22-1.47) for women and men, respectively. GDF-15 and cTnI had a similar relationship with MACE with overlapping confidence intervals in both sexes (women: HR 1.49 [95%CI 1.35-1.60] and HR 1.42 [95%CI 1.27-1.58], men: HR 1.34 [95%CI 1.22-1.47] and HR 1.24 [95%CI 1.13-1.37]. In contrast, the relationship with MACE was weaker for cTnT and CRP compared to NT-proBNP.

Combining biomarkers for the prediction of cardiovascular events

Discrimination of the base model using SCORE2 risk factors was excellent for both women and men (c-indices 0.826 and 0.795, respectively). As compared with the baseline model, GDF-15 improved the c-index by +0.010 with an IDI of 0.015 for women (***Fig. 3, Supplemental Table 4-7***). For women, combined biomarker models that included GDF-15 together with NT-proBNP (+0.012), or GDF-15 plus cTnI (+0.013), but not CRP or cTnT, further improved the c-index. As compared with the baseline model, NT-proBNP improved the c-index by +0.005 with an IDI of 0.014 for men (***Fig. 3, Supplemental Table 4-7***). For men, combined biomarker models that included NT-proBNP together with GDF-15 (+0.007), NT-proBNP plus cTnI (+0.006), and NT-proBNP plus CRP (+0.008), but not cTnT, further improved the c-index. The

greatest numerical improvement in discrimination from the base model was achieved with NT-proBNP, GDF-15 and cTnI for women (+0.014) and NT-proBNP, GDF-15, cTnI and CRP for men (+0.010) (***Fig. 3***). The combined model incorporating NT-proBNP, GDF-15, and cTnI yielded an IDI of +0.033 and a continuous NRI of +0.254 in women (***Supplemental Table 6 and 8***). The combined model incorporating NT-proBNP, GDF-15, cTnI and CRP yielded an IDI of +0.017 and a continuous NRI of +0.120 in men (***Supplemental Table 6 and 9***). Generally, cardiac biomarkers improved risk classification among cases more than non-cases.

The base SCORE2 models performed better in individuals aged <40 years as compared to those aged ≥40 years (c-index 0.831 versus 0.766, ***Supplemental Table 10***). Although discriminative performance was weaker in those aged ≥40 years, this group had the greatest improvement in the c-index with the addition of NT-proBNP and GDF-15. For NT-proBNP, the change in c-index, as compared with the base model, was +0.001 and +0.008 in individuals aged <40 years and ≥40 years, respectively. For GDF-15, the change in c-index, as compared with the base model, was +0.000 and +0.0010 in individuals aged <40 years and ≥40 years, respectively. Conversely, cTnI showed greatest improvement in individuals aged <40 years as compared to their counterparts (change in c-index: +0.013 *versus* +0.006). A combined biomarker model that included NT-proBNP, GDF-15 and cTnI yielded a categorical NRI of +0.048 for individuals ≥40 years (***Supplemental Table 11***).

Secondary analysis

Our results did not change when we set cTnI and cTnT concentrations below the LoD at the LoD/2 value (***Supplemental Table 12***). We observed a similar pattern in the improvement of discrimination, relative to a base model that also included socioeconomic status, for single and combined biomarker models (***Supplemental Table 13***). In addition, we evaluated the

association of creatinine with primary outcome. Compared with NT-proBNP, the association with creatinine was weaker (***Supplemental Table 14***). After adjustment, we found that higher creatinine was associated with MACE in women (HR 1.16 [95%CI 1.06 to1.28]) but not in men (HR 1.06 [95%CI 0.96 to 1.17]). When evaluating biomarkers for secondary outcome, we observed that NT-proBNP was numerically more strongly associated with myocardial infarction than either cTnT or cTnI in crude models, but similar associations were found in adjusted models (***Supplemental Table 15***). NT-proBNP and cTnI were not associated with non-cardiovascular death in adjusted models for women, although GDF-15, cTnT and CRP were associated with non-cardiovascular death.

Discussion

We evaluated multiple cardiac biomarkers to predict MACE in a large population-based cohort study. Our main finding was that combining cardiac biomarkers, particularly NT-proBNP, GDF-15 or cTnI, improved estimates of cardiovascular risk over a base model using traditional SCORE2 risk factors in both women and men.

Our study has several strengths. First, we used a large contemporary population-based cohort study of >18,000 individuals with more than 10 years of follow-up. Second, the large number of women and men over a wide age range included in this study allowed us to conduct a sex- and age-specific analysis. Third, we were able to measure five candidate biomarkers for the prediction of cardiovascular risk in GS:SFHS. This enabled us to perform a systematic evaluation of combined biomarker approaches for cardiovascular risk prediction. Finally, NT-proBNP, cTnT, cTnI and CRP were measured using assays that are commonly used in clinical

biochemistry services around the world, with CRP, cTnT and cTnT measured by high-sensitivity assays.

A number of studies have evaluated the ability of circulating cardiac biomarkers to predict cardiovascular disease in populations of presumably healthy individuals (13-16, 30-34). Recently, Wu *et al.* evaluated the use of multiple circulating biomarkers in addition to the PREDICT risk factors, and also found that the addition of NT-proBNP and cardiac troponins refined cardiovascular risk estimates (32). Similarly, the ULSALM study of 826 older men, using a research use only proteomics approach, reported NT-proBNP to be the biomarker most strongly associated with cardiovascular disease (35). In line with previous reports, we observed that NT-proBNP, traditionally considered a biomarker of heart failure, was strongly associated with MACE. NT-proBNP was particularly strongly additive to the risk score in those aged ≥40 years, an age at which risk prediction models are more often applied in clinical practice. Given increasing interest in using NT-proBNP in some patient groups to screen for heart failure in the absence of signs and symptoms of the condition (36), these collective findings highlight the advantages of prioritizing NT-proBNP for incorporation in commonly applied cardiovascular risk scores. Although our findings are complementary, we provide additional insights by inclusion of two additional cardiac biomarkers, cTnT and GDF-15, because recent studies have shown both are independently associated with future cardiovascular events in the general population (3, 9, 37, 38). Our findings show that GDF-15 should also be considered for cardiovascular risk assessment (32). Blankenberg *et al.* previously assessed 30 candidate biomarkers in relation to cardiovascular risk prediction in a smaller study (n=7915) and found that NT-proBNP, cTnI and CRP when added to established risk factors improved performance when compared to a baseline model in men (13). We extended current knowledge by conducting a comprehensive sex- and age-specific analysis, and showed that NT-proBNP,

combined particularly with GDF-15 or cTnI, showed greatest improvement in prediction of cardiovascular risk as compared with a baseline model for both women and men and all age groups. A biomarker-driven strategy that uses NT-proBNP combined with GDF-15 or cTnI may contribute to further improvement in cardiovascular risk assessment.

Sex disparities in primary prevention and treatment of cardiovascular disease exists (39, 40), and using a biomarker-driven risk assessment approach may reduce the gap between women and men. In line with previous studies (17-19), we observed important sex-differences in cardiac biomarker concentrations and in their association with MACE. While for NT-proBNP, GDF-15 and CRP higher baseline concentrations were observed in women, higher cTnI and cTnT concentrations were observed in men. We also found that the association between all cardiac biomarkers and MACE was numerically stronger in women than men, but this divergence diminished after adjustment for cardiovascular risk factors. These observations are particularly important with respect to integration of cardiac biomarkers in our cardiovascular risk estimation systems. A binary approach that uses a uniform cardiac biomarker threshold will not contribute to reduce current inequalities, but rather may increase the existing gap. In previous research we showed that age and other cardiovascular risk factors like diabetes and body mass index are important modifying factors between sex, cardiac biomarkers and clinical outcomes (22, 41, 42). Altogether, this indicates that an approach using sex-specific thresholds to predict cardiovascular disease in the primary care setting is also too simplistic. The digitalization of electronical health records enables the opportunity to embed cardiovascular risk estimation systems that includes cardiac biomarkers as a continuous variable together with other cardiovascular risk factors and preventative therapies for use in clinical practice. Evaluation of implementation of biomarker-driven risk assessment tools in practice is required and an important step to assess the impact of these tools on care for women and men.

Our findings suggests that cTnT, CRP and creatinine are the weakest independent predictors in a presumably healthy population and are less useful for cardiovascular risk assessment. Although the underlying mechanisms are not well understood, cTnT seems to be more strongly associated with non-cardiovascular disease like chronic kidney disease and muscular disease than cTnI (43, 44). We also found that CRP marginally improved risk prediction for men but not for women. This is in line with the findings of a large study that included 246,669 individuals who were presumed to be healthy, which showed that the change in c-index for CRP when added to a base model was +0.0077 (+0.0058 to 0.0096) for men and +0.0007 (-0.0007 to 0.0021) for women (45). Altogether, this indicates that the use of cTnT and CRP for cardiovascular risk estimation may be less incrementally beneficial than other cardiac biomarkers. It should be noted that CRP has been added to the secondary prevention SMART2 risk score (46).

In this study we report that a model including established cardiovascular risk factors performed well with excellent discrimination and a c-index of ~0.8 for both women and men. This is likely at least in part due to the wide age range of our cohort. This level of discrimination in a risk score makes it very difficult to demonstrate incremental value with the addition of cardiac biomarkers, and explain the modest increments in the C-statistic that they determined. Despite this, our data indicates that the use of NT-proBNP, GDF-15 and cTnI provide additional prognostic information not captured currently by established risk factors. The NRI suggests improved risk classification among cases, which would lead to more appropriate and intensive treatments for those who require it most. The complementary information provided by these disease-specific biomarkers can therefore further enhance patient and clinician understanding of the impact of risk factors on the cardiovascular system and may help target interventions to

those individuals who are at high risk of a future cardiovascular event. For incorporation of cardiac biomarkers into cardiovascular risk scores, it should be taken into account that the gain of adding cardiac biomarkers to risk scores seems highest for individuals aged ≥40 years. Other options for refining cardiovascular risk include the use of coronary artery calcification (CAC) score. A recent meta-analysis suggested that the c-index of a base model was improved by +0.036 with the addition of the CAC score, although the base model performance was lower in this study (range: 0.693-0.800) and heterogeneity of the estimated improvement in discrimination was high (47). To get better understanding on the clinical implications of our study, additional research is needed on the costs, risks and benefits of using combined cardiac biomarkers or CAC scoring for cardiovascular risk refinement.

Our study has several limitations. First, the GS:SFHS cohort includes predominantly White individuals, limiting generalizability of our findings to other ethnicities. Second, our analysis is restricted to two manufacturers' assays and direct extrapolation of our findings to other manufacturers' assay cannot be made. Third, biomarker measurements were only available at one point in time; we could not evaluate the relationship between biomarker trajectories and cardiovascular risk. Finally, we have used the SCORE2 outcome of MACE that does not include heart failure. Future research should evaluate the ability of biomarkers to predict the onset of heart failure, which may be the first manifestation of cardiovascular disease.

In conclusion, cardiac biomarkers - particularly NT-proBNP, GDF-15 and cTnI - further refined cardiovascular risk estimates compared to a currently recommended model using traditional risk factors. A biomarker-driven strategy that uses NT-proBNP combined with GDF-15 or cTnI may contribute to further improvement in cardiovascular risk assessment for prevention of cardiovascular disease in women and men.

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Conflict of interest

PW reports grant income from Roche Diagnostics in relation to and outside of the submitted work, as well as grant income from AstraZeneca, Boehringer Ingelheim, and Novartis, outside the submitted work and speaker fees from Novo Nordisk, and Raisio outside the submitted work. DAG has received consultancy fees from Optima Partners. REM has received speaker fees from Illumina and is an advisor to the Epigenetic Clock Development Foundation and Optima Partners. MW has recently been a consultant to Amgen and Freeline. ASVS's institution has received honoraria from Abbott Diagnostics outside the submitted work. JGFC has received research funding and personal honoraria from Bristol Myers Squibb, Medtronic,

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Author Contributions

All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

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Table 1. Baseline characteristics of study participants with and without incident MACE on follow up

	All (n=18,383)	No incident MACE (n=17,441)	Incident MACE (n=942)
Age (years)	48 (35 to 58)	47 (35 to 57)	61 (54 to 69)
Sex (male)	7,553 (41.1%)	7,025 (40.3%)	528 (56.1%)
Body mass index (kg/m^2)	26.6 (5.2)	26.5 (5.1)	28.0 (5.2)
Systolic blood pressure (mmHg)	131 (18)	131 (17)	142 (20)
Total cholesterol (mmol/L)	5.1 (1.1)	5.1 (1.1)	5.4 (1.2)
High-density lipoprotein cholesterol (mmol/L)	1.5 (0.4)	1.5 (0.4)	1.4 (0.4)
SIMD (score/10)	1.2 (0.7 to 2.2)	1.1 (0.7 to 2.2)	1.3 (0.7 to 2.5)
eGFR (ml/min/1.73m^2)	96 (17)	96 (17)	83 (18)
Current smoking (yes)	2,883 (16.2%)	2,696 (16.0%)	187 (20.8%)
Family history of CVD (yes)	6,966 (38.7%)	6,589 (38.6%)	377 (40.9%)
Diabetes Mellitus (yes)	433 (2.4%)	360 (2.1%)	73 (7.7%)
Lipid modifying medication (yes)	931 (5.1%)	798 (4.6%)	133 (14.1%)
Antihypertensive medication (yes)	1,270 (6.9%)	1,093 (6.3%)	177 (18.8%)
NT-proBNP (pg/mL)	50.6 (26.4 to 91.5)	49.4 (26.0 to 88.8)	78.5 (40.7 to 174.3)
GDF-15 (pg/mL)	807.0 (608.1 to 1103.0)	791.1 (601.3 to 1072.0)	1241.0 (884.4 to 1799.8)
cTnI (ng/L)	1.9 (0.6 to 3.0)	1.8 (0.6 to 2.9)	2.9 (2.0 to 5.1)
cTnT (ng/L)	3.2 (1.5 to 5.8)	3.1 (1.5 to 5.6)	5.7 (1.5 to 9.8)
CRP (mg/L)	1.2 (0.6 to 2.8)	1.2 (0.6 to 2.7)	1.8 (0.9 to 3.9)

Continuous variables are presented as mean (SD) or median (25th to 75th percentile), as appropriate. Categorical variables are presented as number (%). Abbreviations: SIMD, Scottish Index Multiple Deprivation score; eGFR, estimated Glomerular Filtration Rate; CVD, cardiovascular disease; N-terminal pro-B-type natriuretic peptide, NT-proBNP; growth differentiation factor-15, GDF-15; cardiac troponin I, cTnI; cardiac troponin T, cTnT; C-reactive protein, CRP; MACE, major adverse cardiovascular events. Missing values < 5% if applicable, except for SIMD (5.7%).

Table 2. Association of biomarkers (per 1 SD increase on the log_2 scale) with MACE in separate models

	NT-proBNP	GDF-15	cTnI	cTnT	CRP
Women	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Crude model	2.62 (2.37 to 2.90)	2.18 (2.04 to 2.32)	1.90 (1.78 to 2.03)	2.11 (1.93 to 2.31)	1.51 (1.37 to 1.66)
Adjusted model*	1.56 (1.38 to 1.75)	1.49 (1.35 to 1.66)	1.42 (1.27 to 1.58)	1.28 (1.15 to 1.43)	1.27 (1.15 to 1.41)
Ratio of Biomarker HR:NT-proBNP HR†	Reference	0.96 (0.85 to 1.06)	0.91 (0.80 to 1.02)	0.82 (0.71 to 0.93)	0.82 (0.71 to 0.92)
Men	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Crude model	2.04 (1.89 to 2.20)	1.82 (1.72 to 1.93)	1.49 (1.39 to 1.60)	1.56 (1.43 to 1.70)	1.50 (1.38 to 1.63)
Adjusted model*	1.34 (1.22 to 1.47)	1.24 (1.13 to 1.37)	1.21 (1.10 to 1.32)	1.04 (0.94 to 1.15)	1.17 (1.07 to 1.29)
Ratio of Biomarker HR:NT-proBNP HR†	Reference	0.93 (0.83 to 1.02)	0.90 (0.80 to 0.99)	0.77 (0.67 to 0.87)	0.87 (0.78 to 0.97)

* Models are adjusted for SCORE2 risk factors: age, smoking status, systolic blood pressure, diabetes mellitus, total cholesterol and high-density lipoprotein. Abbreviations: N-terminal pro-B-type natriuretic peptide, NT-proBNP; growth differentiation factor-15, GDF-15; cardiac troponin I, cTnI; cardiac troponin T, cTnT; C-reactive protein, CRP; MACE, major adverse cardiovascular events; HR, hazard ratio.

† Hazard ratio for the specified biomarker (adjusted model) divided by the hazard ratio for NT-proBNP (adjusted model). This model tests which of the biomarkers have evidence of stronger or weaker adjusted associations with MACE compared to NT-proBNP.

Figure legends.

Fig. 1. Crude association of N-terminal pro-B-type natriuretic peptide (NT-proBNP), GDF-15 (growth differentiation factor-15), cardiac troponin T (cTnT), cardiac troponin I (cTnI) and C-reactive protein (CRP) with major adverse cardiovascular events (MACE).

Fig. 2. Adjusted association of N-terminal pro-B-type natriuretic peptide (NT-proBNP), GDF-15 (growth differentiation factor-15), cardiac troponin T (cTnT), cardiac troponin I (cTnI) and C-reactive protein (CRP) with major adverse cardiovascular events (MACE). Models are adjusted for SCORE2 risk factors: age, smoking status, systolic blood pressure, diabetes mellitus, total cholesterol and high-density lipoprotein cholesterol.

Fig. 3. Improved discrimination of major adverse cardiovascular events (MACE), relative to a baseline model, of single and combined biomarker models in women and men. Baseline model included SCORE2 risk factors: age, smoking status, systolic blood pressure, diabetes mellitus, total cholesterol and high-density lipoprotein cholesterol.