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Multiple cardiac biomarkers to improve prediction of cardiovascular events

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

7
8 **Running title:** Combined biomarker approach & CVD

9
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1 **Nonstandard abbreviations**

2

3 **NT-proBNP** N-terminal pro-B-type natriuretic peptide

4 **GDF-15** growth differentiation factor-15

5 **cTnI** cardiac troponin I

6 **cTnT** cardiac troponin T

7 **CRP** C-reactive protein

8 **MACE** major adverse cardiovascular events

9 **GS:SFHS** Generation Scotland Scottish Family Health study

10 **STROBE** Strengthening the Reporting of Observational Studies in Epidemiology

11 **LoD** limit of detection

12 **LoB** limit of blank

13 **UKNEQAS** National External Quality Assurance Scheme

14 **ICD-10** 10th revision of the International Classification of Diseases

15 **IDI** integrated discrimination index

16 **NRI** net reclassification index

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1 **Abstract**

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

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

13 **Results:** The study population comprised 18,383 individuals (58.9% women, median age of
14 48 years [25th-75th percentile, 35-58 years]). During the median follow up of 11.6 (25th-75th
15 percentile, 10.8-13.0) years, MACE occurred in 942 (5.1%) individuals. The greatest increase
16 in discrimination with addition of individual biomarkers to base model was for women GDF-
17 15 and for men NT-proBNP (change in c-index: +0.010 for women and +0.005 for men). For
18 women, combined biomarker models that included GDF-15 and NT-proBNP (+0.012) or
19 GDF-15 and cTnI (+0.013), but not CRP or cTnT, further improved discrimination. For men,
20 combined biomarker models that included NT-proBNP and GDF-15 (+0.007), NT-proBNP
21 and cTnI (+0.006), or NT-proBNP and CRP (+0.008), but not cTnT, further improved
22 discrimination.

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

25 **Keywords:** Biomarkers, cardiovascular, risk factors, general population

26

1 **Introduction**

2

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

8

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

23

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

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

5

6 **Methods**

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

10

11 **Study population**

12 The GS:SFHS is a family-based cohort that enrolled 24,090 participants aged between 18 and
13 98 years (20, 21). Briefly, individuals between 35 and 65 years old were identified at random
14 from participating general practices in Scotland between February 2006 and March 2011.
15 Participants were then asked to identify one or more first-degree relatives ≥ 18 years old who
16 would also be able to participate. For this study, we excluded participants with cardiovascular
17 disease at baseline, those who had missing cardiac biomarker measurements, or who did not
18 attend the clinical survey. As GDF-15 concentrations are temporarily substantially increased
19 during pregnancy, we also excluded pregnant women (self-reported or when GDF-15
20 concentrations were $>10,000$ pg/mL in women aged ≤ 45 years) (22). Participants completed a
21 health questionnaire, and clinical characteristics were measured using a standardized protocol.
22 The cohort is almost entirely of White ethnicity (99%) (20) and therefore is not further reported
23 in this study. Study participants provided written informed consent, including linkage to their
24 medical records. Ethical approval for the GS:SFHS study was obtained from the National
25 Health Service Tayside Research Ethics Committee (Research Ethics Committee reference

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

4

5 **Biomarker measurements**

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

23

24 **Clinical outcomes**

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

11

12 **Statistical analysis**

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

18

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

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

8

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

20

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

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

8

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

14

15 **Results**

16

17 **Clinical characteristics of study population**

18 The cohort comprised 18,383 individuals (58.9% women, median age 48 [25th-75th percentile,
19 35-58; range 18-94] years; *Table 1*). Cardiac biomarker concentrations were generally low;
20 15.0% had an NT-proBNP above 125 pg/mL, 0.8% had a GDF-15 >4000 pg/ml, 0.8% had a
21 cTnI >26.2 ng/L, 2.6% had a cTnT >14 ng/L, and 33.9% had a CRP >2 mg/L.

22

23 MACE occurred in 717 (4.0%) of individuals over ten years (*Supplemental Table 2*) and in
24 942 (5.1%) individuals during the total median follow up of 11.6 (25th-75th percentile, 10.8-
25 13.0) years. In both women and men, baseline concentrations of biomarkers were higher in

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

5

6 **The association of circulating biomarkers with cardiovascular events**

7 In unadjusted single biomarker models, NT-proBNP had numerically the strongest and CRP
8 had the weakest association with MACE in both sexes (*Fig. 1-2, Table 2*). After adjusting for
9 conventional risk factors included in the SCORE2 risk equation, the HR of NT-proBNP per 1
10 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
11 women and men, respectively. GDF-15 and cTnI had a similar relationship with MACE with
12 overlapping confidence intervals in both sexes (women: HR 1.49 [95%CI 1.35-1.60] and HR
13 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
14 contrast, the relationship with MACE was weaker for cTnT and CRP compared to NT-proBNP.

15

16 **Combining biomarkers for the prediction of cardiovascular events**

17 Discrimination of the base model using SCORE2 risk factors was excellent for both women
18 and men (c-indices 0.826 and 0.795, respectively). As compared with the baseline model, GDF-
19 15 improved the c-index by +0.010 with an IDI of 0.015 for women (*Fig. 3, Supplemental*
20 *Table 4-7*). For women, combined biomarker models that included GDF-15 together with NT-
21 proBNP (+0.012), or GDF-15 plus cTnI (+0.013), but not CRP or cTnT, further improved the
22 c-index. As compared with the baseline model, NT-proBNP improved the c-index by +0.005
23 with an IDI of 0.014 for men (*Fig. 3, Supplemental Table 4-7*). For men, combined biomarker
24 models that included NT-proBNP together with GDF-15 (+0.007), NT-proBNP plus cTnI
25 (+0.006), and NT-proBNP plus CRP (+0.008), but not cTnT, further improved the c-index. The

1 greatest numerical improvement in discrimination from the base model was achieved with NT-
2 proBNP, GDF-15 and cTnI for women (+0.014) and NT-proBNP, GDF-15, cTnI and CRP for
3 men (+0.010) (**Fig. 3**). The combined model incorporating NT-proBNP, GDF-15, and cTnI
4 yielded an IDI of +0.033 and a continuous NRI of +0.254 in women (**Supplemental Table 6**
5 **and 8**). The combined model incorporating NT-proBNP, GDF-15, cTnI and CRP yielded an
6 IDI of +0.017 and a continuous NRI of +0.120 in men (**Supplemental Table 6 and 9**).
7 Generally, cardiac biomarkers improved risk classification among cases more than non-cases.
8
9 The base SCORE2 models performed better in individuals aged <40 years as compared to those
10 aged ≥ 40 years (c-index 0.831 versus 0.766, **Supplemental Table 10**). Although discriminative
11 performance was weaker in those aged ≥ 40 years, this group had the greatest improvement in
12 the c-index with the addition of NT-proBNP and GDF-15. For NT-proBNP, the change in c-
13 index, as compared with the base model, was +0.001 and +0.008 in individuals aged <40 years
14 and ≥ 40 years, respectively. For GDF-15, the change in c-index, as compared with the base
15 model, was +0.000 and +0.0010 in individuals aged <40 years and ≥ 40 years, respectively.
16 Conversely, cTnI showed greatest improvement in individuals aged <40 years as compared to
17 their counterparts (change in c-index: +0.013 *versus* +0.006). A combined biomarker model
18 that included NT-proBNP, GDF-15 and cTnI yielded a categorical NRI of +0.048 for
19 individuals ≥ 40 years (**Supplemental Table 11**).

20

21 **Secondary analysis**

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

1 association of creatinine with primary outcome. Compared with NT-proBNP, the association
2 with creatinine was weaker (*Supplemental Table 14*). After adjustment, we found that higher
3 creatinine was associated with MACE in women (HR 1.16 [95%CI 1.06 to 1.28]) but not in men
4 (HR 1.06 [95%CI 0.96 to 1.17]). When evaluating biomarkers for secondary outcome, we
5 observed that NT-proBNP was numerically more strongly associated with myocardial
6 infarction than either cTnT or cTnI in crude models, but similar associations were found in
7 adjusted models (*Supplemental Table 15*). NT-proBNP and cTnI were not associated with non-
8 cardiovascular death in adjusted models for women, although GDF-15, cTnT and CRP were
9 associated with non-cardiovascular death.

10

11 **Discussion**

12

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

17

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

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

3

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

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

5
6 Sex disparities in primary prevention and treatment of cardiovascular disease exists (39, 40),
7 and using a biomarker-driven risk assessment approach may reduce the gap between women
8 and men. In line with previous studies (17-19), we observed important sex-differences in
9 cardiac biomarker concentrations and in their association with MACE. While for NT-proBNP,
10 GDF-15 and CRP higher baseline concentrations were observed in women, higher cTnI and
11 cTnT concentrations were observed in men. We also found that the association between all
12 cardiac biomarkers and MACE was numerically stronger in women than men, but this
13 divergence diminished after adjustment for cardiovascular risk factors. These observations are
14 particularly important with respect to integration of cardiac biomarkers in our cardiovascular
15 risk estimation systems. A binary approach that uses a uniform cardiac biomarker threshold
16 will not contribute to reduce current inequalities, but rather may increase the existing gap. In
17 previous research we showed that age and other cardiovascular risk factors like diabetes and
18 body mass index are important modifying factors between sex, cardiac biomarkers and clinical
19 outcomes (22, 41, 42). Altogether, this indicates that an approach using sex-specific thresholds
20 to predict cardiovascular disease in the primary care setting is also too simplistic. The
21 digitalization of electronic health records enables the opportunity to embed cardiovascular
22 risk estimation systems that includes cardiac biomarkers as a continuous variable together with
23 other cardiovascular risk factors and preventative therapies for use in clinical practice.
24 Evaluation of implementation of biomarker-driven risk assessment tools in practice is required
25 and an important step to assess the impact of these tools on care for women and men.

1

2 Our findings suggests that cTnT, CRP and creatinine are the weakest independent predictors in
3 a presumably healthy population and are less useful for cardiovascular risk assessment.

4 Although the underlying mechanisms are not well understood, cTnT seems to be more strongly
5 associated with non-cardiovascular disease like chronic kidney disease and muscular disease

6 than cTnI (43, 44). We also found that CRP marginally improved risk prediction for men but
7 not for women. This is in line with the findings of a large study that included 246,669

8 individuals who were presumed to be healthy, which showed that the change in c-index for
9 CRP when added to a base model was +0.0077 (+0.0058 to 0.0096) for men and +0.0007 (-

10 0.0007 to 0.0021) for women (45). Altogether, this indicates that the use of cTnT and CRP for
11 cardiovascular risk estimation may be less incrementally beneficial than other cardiac

12 biomarkers. It should be noted that CRP has been added to the secondary prevention SMART2
13 risk score (46).

14

15 In this study we report that a model including established cardiovascular risk factors performed
16 well with excellent discrimination and a c-index of ~0.8 for both women and men. This is likely

17 at least in part due to the wide age range of our cohort. This level of discrimination in a risk
18 score makes it very difficult to demonstrate incremental value with the addition of cardiac

19 biomarkers, and explain the modest increments in the C-statistic that they determined. Despite
20 this, our data indicates that the use of NT-proBNP, GDF-15 and cTnI provide additional

21 prognostic information not captured currently by established risk factors. The NRI suggests
22 improved risk classification among cases, which would lead to more appropriate and intensive

23 treatments for those who require it most. The complementary information provided by these
24 disease-specific biomarkers can therefore further enhance patient and clinician understanding

25 of the impact of risk factors on the cardiovascular system and may help target interventions to

1 those individuals who are at high risk of a future cardiovascular event. For incorporation of
2 cardiac biomarkers into cardiovascular risk scores, it should be taken into account that the gain
3 of adding cardiac biomarkers to risk scores seems highest for individuals aged ≥ 40 years. Other
4 options for refining cardiovascular risk include the use of coronary artery calcification (CAC)
5 score. A recent meta-analysis suggested that the c-index of a base model was improved by
6 +0.036 with the addition of the CAC score, although the base model performance was lower in
7 this study (range: 0.693-0.800) and heterogeneity of the estimated improvement in
8 discrimination was high (47). To get better understanding on the clinical implications of our
9 study, additional research is needed on the costs, risks and benefits of using combined cardiac
10 biomarkers or CAC scoring for cardiovascular risk refinement.

11

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

20

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

1

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16

17 **Conflict of interest**

18 PW reports grant income from Roche Diagnostics in relation to and outside of the submitted
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16

17 **Author Contributions**

18 All authors confirmed they have contributed to the intellectual content of this paper and have
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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 log₂ 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.