Circulating cardiac biomarkers improve risk stratification for incident cardiovascular disease in community dwelling populations

Zhenqiang Wu,a Anna P. Pilbrow,b Oi Wah Liew,c Jenny P.C. Chong,a John Sluyter,a Lynley K. Lewis,b Moritz Lasse,b Chris M. Frampton,b Rod Jackson,a Katrina Poppe,a Carlos Arturo CamargoJr,d Vicky A. Cameronb,1 Robert Scragg,a,1 and A. Mark Richardsb,c,1*

aSchool of Population Health, University of Auckland, Auckland, New Zealand
bChristchurch Heart Institute, University of Otago, New Zealand
cCardiovascular Research Institute, National University of Singapore, Singapore
dDepartment of Emergency Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Summary

Background Plasma cardiac markers may assist in prediction of incident cardiovascular disease.

Methods The incremental value of cardiac Troponins (T and I) and NT-proBNP added to risk factors in the PRE-DICT score for incident cardiovascular disease (CVD) in primary care, was assessed in 4102 asymptomatic participants in a randomised controlled trial of Vitamin D (ViDA). Findings were corroborated in 2528 participants in a separate community-based observational registry of CVD-free volunteers (HVOLS).

Findings Hazard ratios for first cardiovascular events adjusted for PREDICT risk factors, comparing fifth to first quintiles of marker plasma concentrations, were 2.57 (95% CI 1.47-4.49); 3.01 (1.66-5.48) and 3.38 (2.04-5.60) for hs-cTnI, hs-cTnT and NT-proBNP respectively. The C statistic for discrimination of the primary endpoint increased from 0.755 to 0.771 (+0.016, p = 0.01). Cardiac marker data correctly reclassified risk upwards in 6.7% of patients and downwards in 3.3%. These findings were corroborated by results from HVOLS.

Interpretation Increments in plasma cardiac biomarkers robustly and reproducibly predicted increased hazard of incident CVD, independent of established risk factors, in two community-dwelling populations. Cardiac markers may augment risk assessment for onset of CVD in primary care.

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Introduction

Widely used equations for cardiovascular disease (CVD) risk assessment, including the 2013 American College of Cardiology/American Heart Association Pooled Cohort Equations (PCEs), use data generated from cohorts recruited more than 20 years ago. Among more than 360 models identified on recent review, most were developed in North America and Europe in samples that differ from contemporary community-dwelling populations. The recently published PRE-DICT data provide updated risk equations derived from observation of 401,752 community-dwelling New Zealanders followed for a mean period of 4.2 years with 15,386 (4%) incurring CVD events. PRE-DICT scores incorporate thirteen elements including sex, age, ethnicity, family history of premature CVD, smoking status,
Articles

Research in context

Evidence before this study

Prior to the current study there has been no report to our knowledge of the additive predictive power for incident cardiovascular disease of the circulating cardiac markers, cardiac troponin T, cardiac troponin I and NT-proBNP, as measured by well-validated commercial assays currently in routine clinical use world-wide, when combined with an established panel of cardiovascular risk factors, validated in several hundred thousand community-dwelling people within the last ten years.

Searches on bibliographical databases including Pubmed, Scopus, Embase, Web of Science and Medline using combinations of the terms: incident cardiovascular disease; risk stratification; primary care; circulating cardiac markers; cardiac troponin, NT-proBNP; all filtered for models validated in large populations and published within the period 2010-2020 yielded zero reports.

Added value of this study

Three widely available, standardised and affordable cardiac biomarkers were robustly and independently associated with overall incident cardiovascular disease and with individual categories of adverse cardiovascular events. They added clinically relevant information to a panel of risk factors recently validated in a large community-dwelling population. This combination of circulating markers and risk factors is amenable to rapid and widespread application.

Implications of all the available evidence

Readily measurable circulating cardiac markers add predictive power to well-validated, contemporary tools used to risk stratify for incident cardiovascular disease. Further research should assess the cost-benefit of adding these markers to routine risk assessments in primary care with a view to potentially more effective case selection for added risk surveillance and initiation of cardiovascular pharmacotherapy.

diabetes, systolic blood pressure, total cholesterol:high density lipoprotein cholesterol ratio, social deprivation index, atrial fibrillation, and prescription of blood pressure lowering, lipid-lowering, and antithrombotic drugs. The new equations out-performed the PCES which overestimated risk of atherosclerotic CVD events by 40% in men and 60% in women.1 PREDICT identifies a robust panel of risk factors providing a foundation upon which to evaluate novel candidate predictors.

Circulating biomarkers may independently contribute to cardiovascular risk stratification. The cardiac biomarkers, troponins I and T and amino terminal B type cardiac natriuretic peptide (NT-proBNP) are universally endorsed as aids to diagnosis in acute heart disease.4-6 Over the last 15 years a compelling body of publications has indicated that these markers may also contribute to primary cardiovascular risk stratification. Prior reports include those from Zethelius et al., the “FINRISK97”, Belfast Prospective Epidemiological Study of Myocardial Infarction (PRIME) and “BiomaCaRE” studies, Framingham community study, Atherosclerosis Risk in the Community (ARIC), Womens Health Study, Natriuretic Peptides Collaboration, Cardiovascular Health Study, MONItoring of trends and determinants in CVD (MONICA) study, Risk, Genetics, Archiving, and Monograph (MORGAM) programme, Multi-Ethnic Study of Atherosclerosis (MESA) and others.7-20 Highly sensitive assays for the cardiac troponins and for NT-proBNP are now well standardised, widely available and affordable. We investigated if cardiac biomarker data further improved risk stratification for incident CVD, beyond the established well-validated risk factors included in PREDICT, in two middle-aged to elderly New Zealand cohorts typical of community dwelling people encountered in primary care undergoing screening for CVD risk.

Methods

The incremental predictive performance of cardiovascular biomarkers added to the PREDICT risk factors (sex, age, ethnicity, family history of premature CVD, smoking status, diabetes, systolic blood pressure, total cholesterol:high density lipoprotein cholesterol ratio, social deprivation index, atrial fibrillation, and prescription of blood pressure lowering, lipid-lowering, and antithrombotic drugs) for incident CVD, was assessed in participants in the Vitamin D Assessment (ViDA) Study and further validated in the Canterbury Health Volunteers Study (HVOLS).21,22 The ViDA study, a randomised, double-blind, placebo-controlled trial (Australian New Zealand Clinical Trials Registry ACTRN12611000402943) has been reported in full elsewhere.23 In brief ViDA recruited community-dwelling people in Auckland, NZ. Participants (n = 5110) were randomised to receive vitamin D3 (n = 2558) or placebo (n = 2552). Inclusion criteria were: age 50–84 years; ability to give informed consent with anticipated residence in NZ for the 4-year study period. Exclusion criteria were: current use of vitamin D supplements, psychiatric disorders limiting protocol compliance, hypercalcaemia, nephrolithiasis, sarcoidosis, parathyroid disease or gastric bypass surgery; enrolment in another study or serum calcium >2.50 mmol/L.

Participants gave written informed consent. Data collection included height, weight, blood pressure, sociodemographic status, smoking status, alcohol intake, leisure-time physical activity, sun exposure, intake of vitamin D or calcium supplements, current
medications, and medical history (including hypertension, coronary heart disease, cardiac failure, cardiac arrhythmia, hyperlipidaemia, stroke, venous thrombosis, and diabetes). Information collected in the trial provided the PREDICT risk variables with the exception of family history of premature cardiovascular disease. A 25-mL blood sample was collected at baseline. The study was approved by New Zealand Multi-region Ethics Committee, Wellington (MEC/09/08/082).

Participants in the HVOLS (Trial Registry ACTRN1260500448640) were randomly selected from the Canterbury, NZ electoral rolls. Participants (n = 3358) were 20-108 years with no history of CVD including angina, coronary artery disease, myocardial infarction or peripheral vascular disease. Participants completed a questionnaire on their personal health and medical history, family heart history, smoking status, alcohol consumption and self-reported physical activity. Blood pressure, height, weight, waist and hip measurements were documented. Blood samples were taken at recruitment for neurohormone and genetic analyses. The study was approved by the Upper South A Ethics Committee (Reference No. CTY/01/05/062), and each participant provided written, informed consent. The current report incorporates data in a subset (n = 2528) of the HVOL Study for whom samples were available for biomarker assays.

Immunoaassays
EDTA plasma aliquots were stored at -80°C. NT-proBNP and high sensitivity cardiac troponin T (hs-cTnT) underwent electrochemiluminescence immunoassay on the ELECSYS Cobas 6411 immunoanalyzer (Roche Diagnostics, Basel, Switzerland). Working ranges of NT-proBNP and hs-cTnT assays were 535,000 pg/ml and 3-10,000 pg/ml respectively. Inter-assay coefficients of variation (CoV) for the low (NT-proBNP, 143 pg/ml, 2.64%; hs-cTnT, 26.5 pg/ml, 4.56%) and high (NT-proBNP, 4505 pg/ml, 2.18%; hs-cTnT, 2121 pg/ml, 1.52%) quality control samples were derived from 72 to 42 independent runs for NT-proBNP and hs-cTnT, respectively. The high sensitivity cardiac troponin I (hs-cTnl) was assayed on the Abbott Architect 1200SR analyser (Abbott Laboratories, Illinois, USA). The inter-assay CoV (n = 29) was 5.15% at 21.2 pg/ml, 5.05% at 206 pg/ml and 3.67% at 15,615 pg/ml.

Follow-up and outcomes
For both cohorts, deaths and hospital discharges (classified according to the International Statistical Classification of Diseases and Health Related Problems, Tenth Revision [ICD-10], [Suppl File 1]) were tracked as well as dispensed prescriptions (generic name, dose, and frequency) using participants’ unique New Zealand National Health Index numbers, over follow-up. The primary endpoint was the composite of all first cardiovascular events (Myocardial infarction, Unstable angina, other coronary heart disease, Ischaemic stroke, Haemorrhagic stroke, Transient ischemic attack, Peripheral vascular disease, Congestive heart failure, Other Ischaemic CVD-related deaths, Suppl File 1). Secondary endpoints included all-cause mortality; acute coronary ischemic events; cerebral ischemic events (transient ischemic attacks and cerebrovascular accidents) and acute heart failure. For the current analysis follow-up was limited to 5 years from recruitment or to any earlier relevant first event.

Within the ViDA trial vitamin D had no effect on the main outcomes: CVD, acute respiratory infections, non-vertebral fractures, falls and all cancer. Data from those participants without antecedent CVD (n = 4102) and irrespective of treatment allocation were included in the current analysis.

Statistical analysis
Demographic and clinical characteristics are presented as mean +/- standard deviation for normally distributed continuous variables, median and interquartile range for skewed continuous variables, and as number and percent for categorical variables. Comparisons between those spared and those incurring clinical endpoints were conducted by Student’s T test or Wilcoxon rank-sum test for continuous variables, and Chi-squared tests for categorical variables. Quantile-Quantile Plot and histogram were used to examine the distribution of continuous variables.

We assessed discrimination and calibration of cohort-derived equations incorporating the PREDICT risk factors. Family history of CVD was not captured in ViDA and analyses assume all participants had no such history. Discrimination was assessed by Harrell’s C statistic which accounts for time to event. Calibration was assessed by categorising participants into deciles of predicted 5-year CVD risk and plotted against observed 5-year risk.

Median (interquartile range) plasma concentrations of cardiac biomarkers were compared between participants incurring versus spared incident CVD. Kaplan-Meier event curves were generated for those with marker values above and below (a) established clinical thresholds (defined below); (b) median and (c) per quintile split, for the primary and secondary endpoints with curves compared by log-rank analysis.

Multivariable Cox proportional hazards regression analysis adjusted for PREDICT risk factors generated hazard ratios for primary and secondary endpoints per- (a) biomarker quintile; (b) natural log increment in biomarker (c) according to marker thresholds endorsed for use in acute cardiac disease. The latter comprised troponin thresholds triggering consideration of acute myocardial infarction in symptomatic patients presenting...
urgently (hs-cTnI ≥16pg/ml in women and ≥34pg/ml in men; hs-cTnT ≥14pg/ml for all-comers) and NT-proBNP thresholds including ≥125pg/ml used to trigger investigation of possible non-acute heart failure, ≥300pg/ml a rule-out threshold for acute decompen-sated heart failure in acute breathlessness and ≥1,000 pg/ml, a threshold strongly associated with worse outcomes in chronic heart failure.4–6,25

The incremental discrimination of those destined to incur CVD was assessed by changes in the C-statistic upon addition of marker data to the PREDICT risk factors. Net Reclassification Index (NRI) was calculated for models before and after addition of cardiac marker (natural logarithm) data to PREDICT risk factors.26

ViDA and HVOLS data sets were analysed similarly. The adjusted hazard ratios associated with increments in biomarker levels were compared between the two cohorts using the method of Altman and Bland.27

Role of the funding source
The study funders played no role in study design; the collection analysis or interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

Results
Baseline characteristics, for 4102 ViDA and 2528 HVOLS participants and the subgroups incurring (n = 248 and 227 respectively) or spared subsequent incident CVD, are displayed in Table 1 and Suppl File 2 respectively. Participants incurring cardiovascular events were older and more likely to be prescribed blood pressure lowering, lipid lowering and or anti-thrombotic medications at baseline than those without events.

In both ViDA and HVOLS studies 5-year CVD rates (6.1 and 9.0% respectively) were substantially higher than the 3.2% in men and 2.3% in women observed in the original PREDICT cohort. Predicted versus observed event rates for each risk decile are depicted in Figure 1 and indicate the models are well-calibrated.

Mean plasma concentrations of all three markers (and the proportion with baseline marker values above nominated thresholds clinically applied in acute disease settings) were significantly higher in participants incurring CVD events (Table 2). In ViDA 27% of participants and half of those experiencing events, had NT-proBNP above 125pg/ml. In 21% of those later suffering events NT-proBNP was over 300pg/ml. Near 5% of the overall study population, and 24% of those with events, had plasma hs-cTnT ≥14pg/ml. Results among HVOLS participants were similar (Suppl File 3).

In ViDA the most frequent events were myocardial infarction (n = 61), stroke (n = 43) and heart failure (n = 48). 120 participants died (47 and 73 from cardiovascular and non-cardiovascular causes respectively) during follow up (Suppl File 4). The distribution of events was similar in HVOLS (Suppl File 5).

Figure 2 depicts significant separation of event curves in the ViDA cohort for each marker for the primary endpoint according to marker levels above or below recognised clinically applied thresholds. Analogous curves for all four secondary endpoints, plotted by (a) clinical thresholds, (b) medians and (c) per quintile of marker levels are displayed in Suppl File 6. Significant separation of event curves was observed for these selected divisions of all three markers for all endpoints. Similar findings were observed in HVOLS (Suppl File 7).

In ViDA, multivariable Cox regression analysis for risk of the primary endpoint adjusted for the PREDICT risk factors demonstrated increased hazards for ascending quintiles of each biomarker. Comparing fifth to first quintiles of marker plasma concentrations at baseline, adjusted hazard ratios were 2.57 (95% CI 1.47-4.49); 3.01 (95% CI 1.66-5.48) and 3.38 (95% CI 2.04-5.60) for hs-cTnI, hs-cTnT and NT-proBNP respectively (Suppl File 8). Shifts in hazard per natural log or by clinical threshold yielded significant results for all three markers (Table 3). Analyses in HVOLS yielded similar results. Adjusted hazard ratios comparing 1st and 5th quintiles of each marker were 4.16 (95% CI 2.09-8.30), p < 0.001; 2.76 (95% CI 1.62-4.73), p = 0.001 and 1.90 (95% CI 1.04-3.47), p = 0.04 for hs-cTnI, hs-cTnT and NT-proBNP respectively (Suppl Files 8 and 9). There was no significant interaction between cohort and hazard ratio for the primary endpoint for any biomarker (Suppl File 10).

For secondary endpoints, in ViDA multivariable analyses adjusted for the PREDICT risk factors and analysed according to marker quintile, natural log or clinically applied thresholds (Table 4) indicated significant increments in hazards for selected divisions of marker levels. hs-cTnI was particularly strong in forecasting new acute coronary events whilst hs-cTnT and NT-proBNP performed better than hs-cTnI for cerebrovascular events and all-cause mortality. NT-proBNP stood out as especially strong in the prediction of heart failure. In HVOLS, similar multivariable analyses relating biomarkers to secondary endpoints yielded similar results (Suppl File 11). Interaction analyses indicated that for secondary endpoints hazard ratios delineated by natural log increments in markers did not differ significantly between the two cohorts (Suppl File 12).

Biomarker data improved the C-statistic (Table 5) for prediction of incident CVD amongst ViDA participants from 0.755 (0.725-0.784) to between 0.763 and 0.764 (p = 0.03-0.13) with the addition of any one marker. The C statistic was further strengthened using any pair of
| Risk Factor | All participants (n=4102) | No CVD (n=3854) | New CVD (n=248) | p value |
|------------|--------------------------|----------------|----------------|---------|
| Gender, n (%) |                          |                |                |         |
| Male       | 2252 (54.9)               | 2068 (53.7)    | 184 (74.2)     | <0.001  |
| Age (ys), mean (SD) | 65.2 (8.0)               | 64.9 (7.9)     | 69.2 (8.3)     | <0.001  |
| Age (ys), n (%) |                      |                |                |         |
| 50-59      | 1057 (25.8)               | 1020 (26.5)    | 37 (14.9)      |         |
| 60-69      | 1913 (46.6)               | 1815 (47.1)    | 98 (39.5)      |         |
| 70-79      | 953 (23.2)                | 872 (22.6)     | 81 (32.7)      |         |
| BMI (kg/m²), mean (SD) | 28.2 (5.0)               | 28.1 (4.9)     | 28.7 (5.8)     | 0.15    |
| Ethnicity, n (%) |                       |                |                | 0.92    |
| Chinese/Other Asian | 99 (2.4)                 | 93 (2.4)       | 6 (2.4)        |         |
| European and Other | 3328 (81.2)              | 3127 (81.1)    | 201 (81.0)     |         |
| Indian/Other South Asian | 205 (5.0)                | 193 (5.0)      | 12 (4.8)       |         |
| Maori      | 202 (4.9)                 | 192 (5.0)      | 10 (4.0)       |         |
| Pacific    | 268 (6.5)                 | 249 (6.5)      | 19 (7.7)       |         |
| NZ Dep quintile, mean (SD) | 2.4 (1.5)                | 2.4 (1.5)      | 2.7 (1.5)      | 0.01    |
| Family history of premature CVD *, n (%) |                     |                |                |         |
| Smoking, n (%) |                                   |                |                | <0.001  |
| Never smoker | 2180 (53.2)               | 2066 (53.6)    | 114 (46.0)     |         |
| Ex-smoker | 1670 (40.7)               | 1566 (40.6)    | 104 (41.9)     |         |
| Current smoker | 252 (6.1)                 | 222 (5.8)      | 30 (12.1)      |         |
| Atrial Fibrillation, n (%) | 377 (9.2)                | 335 (8.7)      | 42 (16.9)      | <0.001  |
| Diabetes, n (%) | 56 (1.4)                 | 46 (1.2)       | 10 (4.0)       | 0.002   |
| SBP (mmHg), mean (SD) | 138.8 (18.4)             | 138.2 (18.3)   | 147.5 (19.1)   | <0.001  |
| eGFR (mL/min/1.73m²), mean (SD) | 69.2 (10.1)              | 69.2 (10.0)    | 68.8 (11.6)    | 0.57    |
| TC/HDL ratio, mean (SD) | 3.7 (0.9)                | 3.6 (0.9)      | 3.7 (1.0)      | 0.10    |
| Table 1: Baseline characteristics of ViDA participants. Family history of premature CVD was not collected in the ViDA study; NZ Dep, New Zealand Index of Socioeconomic Deprivation; SBP, Systolic blood pressure; BMI, body mass index; TC/HDL ratio, Total cholesterol to High Density Lipoprotein cholesterol (TC/HDL) ratio; OBPLM, On blood pressure lowering medications six months prior to the blood date; OLLM, On lipid lowering medications six months prior to the blood date; OATM, On antithrombotic medications six months prior to the blood date; T test was used for continuous variable and Chi-squared test was used for categorical variable in this table unless otherwise specified.
Figure 1. Plots of predicted versus observed event rates in successive deciles of risk in (a) ViDA and (b) HVOLS for cohort-specific risk equations derived from multivariable analyses of ViDA and HVOLS data incorporating the PREDICT risk factors.

Table 2 (Continued)

| Biomarker | All participants (n=4102) | non-CVD (n=3854) | CVD* (n=248) | p value  |
|-----------|---------------------------|-----------------|-------------|----------|
| hs-cTnl (pg/ml), median (IQR) | 3.2 (2.3, 4.7) | 3.1 (2.3, 4.6) | 4.6 (3.1, 7.0) | <0.001 |
| hs-cTnT (pg/ml), median (IQR) | 6.3 (4.1, 9.5) | 6.2 (4.0, 9.1) | 9.6 (6.4, 13.6) | <0.001 |
| NT-proBNP (pg/ml), median (IQR) | 69.5 (34.8, 131.8) | 67.6 (33.9, 126.9) | 118.1 (51.5, 276.9) | <0.001 |

**Natural logarithm of the biomarkers**

| Biomarker | All participants (n=4102) | non-CVD (n=3854) | CVD* (n=248) | p value  |
|-----------|---------------------------|-----------------|-------------|----------|
| ln (hs-cTnl), mean (SD) | 1.3 (0.8) | 1.2 (0.7) | 1.7 (1.0) | <0.001 |
| ln (hs-cTnT), mean (SD) | 1.9 (0.6) | 1.8 (0.6) | 2.3 (0.6) | <0.001 |
| ln (NT-proBNP), mean (SD) | 4.2 (1.1) | 4.1 (1.0) | 4.8 (1.2) | <0.001 |

**Quintile of the biomarkers**

| Biomarker | All participants (n=4102) | non-CVD (n=3854) | CVD* (n=248) | p value  |
|-----------|---------------------------|-----------------|-------------|----------|
| hs-cTnl (pg/ml), n (%) | <2.2 | 838 (20.4) | 821 (21.3) | 17 (6.9) | <0.001 |
| 2.2-<2.9 | 868 (21.2) | 837 (21.7) | 31 (12.5) |
| 2.9-<3.7 | 799 (19.5) | 757 (19.6) | 42 (16.9) |
| 3.7-<4.5 | 793 (19.3) | 727 (18.9) | 66 (26.6) |
| 4.5-<5.4 | 804 (19.6) | 712 (18.5) | 92 (37.1) |
| hs-cTnT (pg/ml), n (%) | <3.60 | 817 (19.9) | 801 (20.8) | 16 (6.5) | <0.001 |
| 3.60-<5.32 | 823 (20.1) | 793 (20.6) | 30 (12.1) |
| 5.32-<7.30 | 820 (20.0) | 792 (20.6) | 28 (11.3) |
| 7.30-<10.41 | 818 (19.9) | 752 (19.5) | 66 (26.6) |
| 10.41-<15.60 | 824 (20.1) | 716 (18.6) | 108 (43.5) |
| NT-proBNP (pg/ml), n (%) | <28.83 | 820 (20.0) | 794 (20.6) | 26 (10.5) | <0.001 |
| 28.83-<53.96 | 820 (20.0) | 780 (20.2) | 40 (16.1) |
| 53.96-<87.98 | 821 (20.0) | 783 (20.3) | 38 (15.3) |
| 87.98-<156.00 | 820 (20.0) | 784 (20.3) | 36 (14.5) |
| ≥156.00 | 821 (20.0) | 713 (18.5) | 108 (43.5) |

**Clinically applied biomarker thresholds**

| Biomarker | All participants (n=4102) | non-CVD (n=3854) | CVD* (n=248) | p value  |
|-----------|---------------------------|-----------------|-------------|----------|
| hs-cTnl (pg/ml), n (%) | Women <16 or men <34 | 3996 (97.4) | 763 (97.6) | 233 (94.0) |
| Women ≥16 or men ≥34 | 106 (2.6) | 91 (2.4) | 15 (6.0) |
| hs-cTnT (pg/ml), n (%) | <14 | 3735 (91.1) | 3545 (92.0) | 190 (76.6) | <0.001 |
| ≥14 | 367 (8.9) | 309 (8.0) | 58 (23.4) |
markers with maximal improvement observed on inclusion of all 3 markers, to 0.771 (0.740-0.801; \( p = 0.01 \)). Similarly, in HVOLS, the C-statistic was significantly improved from 0.777 (0.751-0.803) by addition of any one marker to between 0.784 and 0.792 (\( p = 0.014-0.006 \)) and maximally improved to 0.794 (0.768-0.819; \( p = 0.017 \)) by incorporation of all three markers in the risk equation (Suppl File 13).

Net Reclassification (NRI) observed when biomarker data were added to risk calculated via cohort-specific equations using PREDICT risk factors, yielded correct reclassification upwards of 16/248 cases (6.7%) with incident CVD and correct downward reclassification of 127/3854 (3.3%) cases without incident CVD (Table 6). In the HVOLS data (Suppl File 14) biomarkers yielded 0.5% incorrect upward and 8.7% correct downward reclassification of those incurring or spared incident CVD respectively.

**Discussion**

The key point of distinction in our report lies in assessment of the addition of three widely available, standardised and affordable biomarkers to a panel of risk factors with robust contemporary validation assessed in CVD-free middle-aged to elderly New Zealanders typical of community dwelling people encountered in primary care undergoing screening for risk of incident CVD. This combination of markers and risk factors is amenable to rapid and widespread application. Cardiac biomarkers were robustly and independently associated with overall incident CVD and individual categories of adverse cardiovascular events in two independent, initially asymptomatic, community-based populations. Cardiac biomarker data improved risk stratification for incident CVD, beyond the established well-validated risk factors included in PREDICT.

In sizable minorities of both cohorts, baseline plasma concentrations of three well-recognized cardiac biomarkers reflecting acute or chronic cardiac injury

| Biomarker | All participants (n=4102) | non-CVD (n=3854) | CVD\(^*\) (n=248) | \( p \) value |
|-----------|---------------------------|------------------|-----------------|----------------|
| NT-proBNP (pg/ml), n (%) | | | | |
| <125 | 2996 (73.0) | 2868 (74.4) | 128 (51.6) | <0.001 |
| 125-299 | 848 (20.7) | 783 (20.3) | 65 (26.2) | |
| 300-1000 | 225 (5.5) | 182 (4.7) | 43 (17.3) | |
| ≥1000 | 33 (0.8) | 21 (0.5) | 12 (4.8) | |

Table 2: Biomarkers in the ViDA study.

\( ^* \) New CVD after baseline; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high sensitivity cardiac troponin T; NT-proBNP, N-terminal pro B-type natriuretic peptide; IQR, interquartile range.

\( ^b \) Wilcoxon rank-sum test was used; T test was used for continuous variable and Chi-squared test was used for categorical variable in this table unless otherwise specified.
| hs-cTnI (pg/ml) | HR$^*$ (95% CI), $p$ | hs-cTnT (pg/ml) | HR$^*$ (95% CI), $p$ | NT-proBNP (pg/ml) | HR$^*$ (95% CI), $p$ |
|----------------|-------------------|-----------------|-------------------|------------------|-------------------|
| Quintile of cardiac biomarkers | | | | | |
| <2.2 | 1.00 | <3.60 | 1.00 | <28.83 | 1.00 |
| 2.2–<2.9 | 1.26 (0.69, 2.9), 0.46 | 3.60–<5.32 | 1.41 (0.76, 2.6), 0.28 | 28.83–<53.96 | 1.72 (1.03, 2.85), 0.04 |
| 2.9–<3.7 | 1.63 (0.91, 2.9), 0.10 | 5.32–<7.30 | 1.11 (0.59, 2.1), 0.75 | 53.96–<87.98 | 1.53 (0.91, 2.58), 0.11 |
| 3.7–<5.4 | 2.15 (1.23, 3.75), 0.01 | 7.30–<10.41 | 2.16 (1.20, 3.8), 0.01 | 87.98–<156.0 | 1.41 (0.82, 2.43), 0.21 |
| ≥5.4 | 2.57 (1.47, 4.49), <0.001 | ≥10.41 | 3.01 (1.66, 5.48), <0.001 | ≥156.00 | 3.38 (2.04, 5.60), <0.001 |
| Type 3 Test | $p$<0.001 | $p$<0.001 | $p$<0.001 | $p$<0.001 | |

Natural logarithm of cardiac biomarkers

| In (hs-cTnI) | 1.42 (1.24, 1.62), <0.001 | In (hs-cTnT) | 2.03 (1.60, 2.59), <0.001 | In (NT-proBNP) | 1.54 (1.34, 1.76), <0.001 |

Clinical meaningful cut-off point of cardiac biomarkers

| Women <16 or men <34 | 1.00 | <14 | 1.00 | <125 | 1.00 |
| Women ≥16 or men ≥34 | 2.29 (1.33, 3.95), 0.003 | ≥14 | 1.89 (1.37, 2.6), <0.001 | 125–299 | 1.52 (1.10, 2.1), 0.01 |
| | | | | 300–1000 | 2.92 (1.96, 4.35), <0.001 |
| | | | | ≥1000 | 5.81 (3.01, 11.23), <0.001 |
| Type 3 test | $p$=0.003 | $p$<0.001 | $p$<0.001 |

Table 3: Multivariable Cox regression analysis relating biomarkers to risk of first cardiovascular event - VIDA.

* Adjusted for risk factors in PREDICT CVD v.2015: hs-cTnI, high sensitivity cardiac troponin I; hs-cTnT, high sensitivity cardiac troponin T; NT-proBNP, N-terminal pro b-type natriuretic peptide; the multivariable Cox regression model included the PREDICT CVD v.2015 risk factors and one cardiac biomarker (hs-cTnI or hs-cTnT or NT-proBNP).

| hs-cTnI (pg/ml) | HR$^*$ (95% CI), $p$ | hs-cTnT (pg/ml) | HR$^*$ (95% CI), $p$ | NT-proBNP (pg/ml) | HR$^*$ (95% CI), $p$ |
|----------------|-------------------|-----------------|-------------------|------------------|-------------------|
| Acute coronary syndromes (n=68) | | | | | |
| Quintile of cardiac biomarker | | | | | |
| <2.2 | 1.00 | <3.60 | 1.00 | <28.83 | 1.00 |
| 2.2–<2.9 | 2.16 (0.57, 8.10), 0.25 | 3.60–<5.32 | 1.58 (0.52, 4.79), 0.42 | 28.83–<53.96 | 2.25 (0.85, 5.94), 0.10 |
| 2.9–<3.7 | 2.79 (0.77, 10.18), 0.12 | 5.32–<7.30 | 1.36 (0.42, 4.33), 0.61 | 53.96–<87.98 | 2.37 (0.89, 6.27), 0.08 |
| 3.7–<5.4 | 3.84 (1.08, 3.63), 0.04 | 7.30–<10.41 | 3.27 (1.13, 9.43), 0.03 | 87.98–<156.00 | 2.19 (0.79, 6.07), 0.13 |
| ≥5.4 | 5.12 (1.45, 18.06), 0.01 | ≥10.41 | 3.76 (1.24, 11.41), 0.02 | ≥156.00 | 3.79 (1.40, 10.28), 0.01 |
| Natural logarithm of cardiac biomarker | | | | | |
| In (hs-cTnI) | 1.62 (1.28, 2.04), <0.001 | In (hs-cTnT) | 2.04 (1.29, 3.23), <0.002 | In (NT-proBNP) | 1.30 (1.00, 1.69), 0.05 |
| Clinical meaningful cut-off point of biomarkers | | | | | |
| Women <16 or men <34 | 1.00 | <14 | 1.00 | <125 | 1.00 |
| Women ≥16 or men ≥34 | 3.56 (1.47, 8.64), 0.01 | ≥14 | 1.98 (1.04, 3.79), 0.04 | 125–299 | 1.58 (0.87, 2.88), 0.14 |
| | | | | 300–1000 | 2.22 (0.97, 5.07), 0.06 |
| | | | | ≥1000 | NA |

Cerebrovascular Events (n=89)

| hs-cTnI (pg/ml) | HR$^*$ (95% CI), $p$ | hs-cTnT (pg/ml) | HR$^*$ (95% CI), $p$ | NT-proBNP (pg/ml) | HR$^*$ (95% CI), $p$ |
|----------------|-------------------|-----------------|-------------------|------------------|-------------------|
| Quintile of cardiac biomarker | | | | | |
| <2.2 | 1.00 | <3.60 | 1.00 | <28.83 | 1.00 |
| 2.2–<2.9 | 1.11 (0.45, 2.75), 0.82 | 3.60–<5.32 | 1.89 (0.66, 5.44), 0.24 | 28.83–<53.96 | 1.77 (0.77, 4.08), 0.18 |
| 2.9–<3.7 | 1.48 (0.62, 3.57), 0.38 | 5.32–<7.30 | 1.52 (0.51, 4.53), 0.45 | 53.96–<87.98 | 1.73 (0.74, 4.06), 0.21 |
| 3.7–<5.4 | 1.86 (0.80, 4.31), 0.15 | 7.30–<10.41 | 3.32 (1.20, 9.14), 0.02 | 87.98–<156.00 | 1.34 (0.54, 3.32), 0.52 |
| ≥5.4 | 2.08 (0.89, 4.89), 0.09 | ≥10.41 | 3.57 (1.25, 10.25), 0.02 | ≥156.00 | 3.07 (1.31, 7.19), 0.01 |
| Type 3 Test | $p$=0.029 | $p$=0.03 | $p$=0.04 |
| Natural logarithm of cardiac biomarkers | | | | | |
| In (hs-cTnI) | 1.23 (0.94, 1.62), 0.13 | In (hs-cTnT) | 1.70 (1.11, 2.60), 0.01 | In (NT-proBNP) | 1.41 (1.12, 1.78), 0.004 |
| Clinical meaningful cut-off point of cardiac biomarkers | | | | | |
| Women <16 or men <34 | 1.00 | <14 | 1.00 | <125 | 1.00 |
| Women ≥16 or men ≥34 | 0.97 (0.23, 4.06), 0.97 | ≥14 | 1.13 (0.60, 2.12), 0.70 | 125–299 | 1.43 (0.84, 2.43), 0.19 |
| | | | | 300–1000 | 2.17 (1.06, 4.44), 0.03 |
| | | | | ≥1000 | 3.78 (1.07, 13.36), 0.04 |

Table 4 (Continued)
Cardiac troponins T and I and haemodynamic overload (NT-proBNP) were frequently increased above established guideline-mandated thresholds customarily used to aid diagnosis of acute coronary syndromes or acute heart failure.\textsuperscript{4,6} In both cohorts, markers were higher in participants destined to incur incident CVD compared with peers spared such events.

When added to cohort-specific equations incorporating the PREDICT risk factors, all 3 biomarkers were independently associated with increased risk of incident CVD whether assessed by quintile, natural log increments or by clinically applied marker thresholds. Upper quintile marker levels were associated with adjusted hazards 2-4 fold those observed in the bottom

| Cardiac Biomarker | Heart Failure Events (n=48) | All-cause Death (n=120) |
|-------------------|-----------------------------|-------------------------|
| Quintile of Cardiac Biomarker | | |
| <2.2             | 1.00                         | 1.00                     |
| 2.2–<2.9         | 0.71 (0.10, 5.16, 0.73)      | 0.98 (0.50, 1.92, 0.95)  |
| 2.9–<3.7         | 1.21 (0.21, 6.95, 0.83)      | 0.95 (0.48, 1.86, 0.88)  |
| 3.7–<5.4         | 2.77 (0.58, 13.08, 0.20)     | 0.89 (0.46, 1.73, 0.72)  |
| ≥5.4             | 4.40 (0.95, 20.45, 0.06)     | 0.71 (0.36, 1.43, 0.34)  |

| Natural logarithm of Cardiac Biomarker | Heart Failure Events (n=48) | All-cause Death (n=120) |
|----------------------------------------|-----------------------------|-------------------------|
| In (hs-cTnI)                           | 1.85 (1.44, 2.37, <0.001)   | ln (hs-cTnI)            |
| In (hs-cTnT)                           | 3.91 (2.34, 6.54, <0.001)   | ln (NT-proBNP)          |

| Clinical meaningful cut-off point of cardiac biomarker | Heart Failure Events (n=48) | All-cause Death (n=120) |
|--------------------------------------------------------|-----------------------------|-------------------------|
| Women <16 or men ≤34                                   | 1.00                         | 1.00                     |
| Women ≥16 or men >34                                   | 2.88 (1.05, 7.94, 0.04)      | 4.48 (2.33, 8.64, <0.001) |

| Model | C statistics | Change in C-statistics | p value |
|-------|--------------|------------------------|---------|
| PREDICT CVD v.2019 risk factors<sup>a</sup> | 0.755 (0.725, 0.784) | REF | |
| PREDICT CVD v.2019 risk factors + ln(hs-cTnI) | 0.763 (0.732, 0.791) | 0.008 | 0.03 |
| PREDICT CVD v.2019 risk factors + ln(hs-cTnT) | 0.763 (0.733, 0.794) | 0.009 | 0.08 |
| PREDICT CVD v.2019 risk factors + ln(NT-proBNP) | 0.764 (0.734, 0.795) | 0.009 | 0.13 |
| PREDICT CVD v.2019 risk factors + ln(hs-cTnI)+ln(hs-cTnT)+ln(NT-proBNP) | 0.771 (0.740, 0.801) | 0.016 | 0.01 |

<sup>a</sup> Adjusted for risk factors in PREDICT CVD v.2019 risk score (see Supplementary File 2); hs-cTnI, high sensitivity cardiac troponin I; hs-cTnT, high sensitivity cardiac troponin T; NT-proBNP, N-terminal pro b-type natriuretic peptide; the multivariable Cox regression model included PREDICT CVD v.2019 risk factors and one cardiac biomarker (hs-cTnI or hs-cTnT or NT-proBNP).
The distribution of cardiac biomarkers, including the proportion of apparently elevated plasma concentrations, we observed, is similar to that previously reported in middle-aged to older community dwelling populations. The prevalence of plasma cardiac troponin concentrations above the 99th reference centile in ViDA was 8.9% for hs-cTnT and 2.6% for hs-cTnI, consistent with a Scottish cohort (n = 19,501) from the general population in which TnT was elevated in 3.6% of men and 7.9% of women. The JUPITER study of participants with no prior CVD (n = 12,956) reported elevated hs-cTnT results in 2.9% of men and 4.1% of women. The proportion of participants with NT-proBNP above 125pg/ml was 27% in our group, comparable to the 30% observed in the Cardiovascular Health Study. In accord, the Natriuretic Peptides Studies Collaboration reported a 75th centile of 130 pg/ml for NT-proBNP among 95,617 participants with no prior history of CVD. Our observed relationships between marker levels and CVD match reports from observational cohorts and marker sub-studies of randomised controlled trials. Blankenberg assessed the performance of 30 candidate biomarkers in several thousand community dwelling participants in the FINRISK97 and Belfast PRIME cohorts. NT-proBNP and hs-cTnI ranked amongst the top markers. When incorporated in a marker score, NT-proBNP and hs-cTnI added independent prognostic information with significant risk reclassification. In the JUPITER trial participants with top tertile hs-cTnI levels were at twice the adjusted risk of a first cardiovascular event compared to those with lower tertile values. This is consistent with the hazard ratio of 2.57 [1.47-4.49] we observed for top versus bottom quintiles of hs-cTnI among ViDA participants.

The association of NT-proBNP with cardiovascular risk within the ViDA population also echoed previous reports. In a comparable Danish population, 50-89 years with no prior history of CVD, the adjusted hazard ratio for a first cardiovascular event associated with baseline NT-proBNP values above the 80th percentile was 3.24 similar to the HR of 3.38 [2.04-5.60] we observed for the top quintile of NT-proBNP in the ViDA population.

The power of NT-proBNP in prediction of heart failure was particularly notable. Levels above 300 pg/ml were associated with an adjusted hazard greater than 10-fold that associated with peptide concentrations.

### Table 6: Net reclassification index (NRI) - (risk factors of PREDICT CVD v.2019 + Natural logarithm of biomarker(s) vs risk factors of PREDICT CVD v.2019) in ViDA.

| CVD risk categories | New biomarker, NRI (95% CI), p |
|---------------------|--------------------------------|
|                     | hs-cTnI                       | hs-cTnT                       | NT-proBNP                      | hs-cTnI+hs-cTnT +NT-proBNP     |
| <5, 5<15, ≥15       | Reclassification upward of people with event (%) | 3.6 (0.7, 7.6), 0.09 | 5.2 (0.0, 10.6), 0.05 | 4.8 (-1.6, 10.9), 0.13 | 6.7 (0.5, 12.9), 0.03 |
|                     | Numbers of people reclassified upwards (out of total N=248) | 8 (-2, 18) | 12 (0, 26) | 11 (-3, 27) | 16 (1, 31) |
|                     | Reclassification downward of people without event (%) | 1.2 (0.3, 2.2), 0.01 | 1.8 (0.7, 3.0), 0.003 | 2.2 (1.0, 3.4), <0.001 | 3.3 (1.9, 4.6), <0.001 |
|                     | Numbers of people reclassified downwards (out of total N=3854) | 46 (11, 84) | 69 (26, 115) | 84 (38, 131) | 127 (73, 177) |

NRI in percentage [%]; hs-cTnI, high sensitivity cardiac troponin I; hs-cTnT, high sensitivity cardiac troponin T; NT-proBNP, N-terminal pro B-type natriuretic peptide; Confidence interval were calculated using 10000 bootstrap replicates.
below 125pg/ml (Table 4). The Atherosclerosis Risk in the Community (ARIC) and Natriuretic Peptides Studies Collaboration studies also reported a particularly strong association of NT-proBNP with incident heart failure in initially asymptomatic cohorts.\(^3\,16\)

Notably, in addition to particular strength with respect to heart failure, NT-proBNP was independently predictive of all cardiovascular events consistent with prior reports in which the peptide remained significantly predictive of coronary and stroke events as well as heart failure.\(^16\) The Natriuretic Peptides Studies Collaboration investigators commented that NT-proBNP added more to estimates of cardiovascular risk than HDL-cholesterol. On Kaplan Meier analysis NT-proBNP associated strongly with all-cause mortality, cerebrovascular events, first cardiovascular events and heart failure whereas the cardiac troponins performed more strongly in prediction of new acute coronary events. These findings fit well with NT-proBNP as a marker signalling the integrated effects of age, hypertension and renal dysfunction as well as myocardial strain of any origin. The cardiac troponins T and I were similarly and strongly predictive of coronary events, possibly reflecting sub-clinical ischaemia and cardiomyocyte injury long preceding any overt coronary syndrome. Levels of all three markers may partly reflect low grade cardiac inflammatory processes occurring in vasculature and myocardium.

The relative difference between those incurring and spared CVD in cTnI and cTnT levels is very similar, but the absolute concentrations for cTnT are much closer to the 99th percentile than for cTnI and the proportion of individuals with troponin levels above the 99th percentile differs substantially between cTnT and cTnI. This challenges the appropriateness of using the 99th percentile as cut-off for risk prediction, especially for cTnI. This choice of thresholds reflects their current widespread familiarity in the context of clinical diagnostic applications in acute presentations. However, reference ranges and optimal thresholds for prognostic application in community-based populations will require further definition of the normal range and the optimal thresholds for incorporation in community risk prediction.

Our findings confirm the strength of troponin and NT-proBNP as independent markers for incident cardiovascular disease. ViDA cohort-specific risk equations using PREDICT risk factors and incorporating hs-cTnI + hs-cTnT + NT-proBNP (Table 6) correctly reclassified risk upwards in 6.7% (p = 0.03) of people with incident CVD and correctly downwards in 3.3% (p<0.001) of the larger number of participants without events.

The new equations based on the well-proven elements of the PREDICT score derive additional strength through consideration of ethnicity, socioeconomic status and the documentation of atrial fibrillation; variables which are not incorporated in many existing models.

Limitations of the current report include the moderate cohort sizes and number of cardiovascular events. We acknowledge the ViDA cohort participants were recruited to a randomized controlled trial (RCT) rather than representative of the generality of community-dwellers. HVOLS participants were randomly selected from the Canterbury electoral role and then sub-selected for absence of prior history of CVD. Participants in a RCT are typically healthier with lower risk for future events than the average community-dweller and accordingly participants in the HVOLS cohort had higher CVD risk than those in the ViDA cohort. Nevertheless, the additional predictive value of cardiac markers added to PREDICT factors remains apparent and comparable in both cohorts. The demographic and clinical characteristics of both ViDA and HVOLS cohorts are entirely concordant with the range of people subject to risk stratification in primary care in New Zealand and elsewhere.

The current analysis is limited to 5-year follow-up. Risk stratification for CVD in New Zealand guidelines addresses 5-year risk so the current analysis is applicable to current clinical practice. Extended follow up data is available for HVOLS and does not alter the overall results. We confined ourselves to 5-year data to allow more ready comparison of the two cohorts.

We also assumed absence of family history of CVD for all participants. The impact of this assumption will be minor as this risk factor is not strongly related to CVD (hazard ratio = 1.05-1.14) and its prevalence in NZ adults is not high (~12%); supported by the finding that the HVOL study did measure this variable yet associations were similar between the two studies (Suppl File 13) and a sensitivity analysis, with inclusion or removal of family history, in HVOLS data indicated no substantive impact upon risk prediction (Suppl File 16). We have not assessed serial biomarker measurements which may add a useful dynamic aspect to risk assessments.\(^15\) We have confined analysis to first cardiovascular events and it is likely markers will also aid prediction of second and subsequent events. A larger data set could better define the relative strengths of the different markers for prediction of different categories of cardiovascular events. In mitigation, our findings are corroborated across two independent cohorts. They are also consistent with results from previous reports generated from well-annotated cohort studies.

**Conclusion**

Cardiac biomarkers were robustly and independently predictive of incident CVD in two separate New Zealand community-dwelling cohorts. The cardiac troponin T and I and/or NT-proBNP data enabled sub-categorization of risk over a two to four-fold range when added to established clinical risk factors. NT-proBNP was a particularly powerful predictor of incident heart failure. In individual cases corrected estimates of risk may influence timing of introduction of guideline mandated pharmacotherapies, attention...
to lifestyle factors and intensity of surveillance. The addition of biomarker data to risk equations derived within populations of interest and incorporating the risk factors included in PREDICT, can refine primary risk stratification for cardiovascular disease.

**Contributors**

All authors read and approved the final version of the manuscript.

The following authors have directly accessed and verified the underlying data, Zhenqiang Wu, Anna P Pilbrow, Chris M Frampton, Robert Scragg and A. Mark Richards. All authors agreed to submission of the manuscript.

Zhenqiang Wu: contributed to the conceptualization, visualization and the first draft; analysis and interpretation of data; contributed to the review and editing.

Anna P Pilbrow: Data curation, data analysis, data interpretation, writing - review and editing.

Oi Wah Liew: Conducted assays and contributed to manuscript.

Jenny P C Chong: performed cardiovascular biomarkers measurement of NT-proBNP, hsTnT and hsTnI on blood samples collected from participants of the Vitamin D Assessment (ViDA) Study and Canterbury Health Volunteers Study (HVOLS).

John Sluyter: data curation, project administration, software, supervision, writing (review & editing).

Lynley K Lewis: reviewed data analysis and validity, review and editing of final manuscript.

Moritz Lasse: data curation; data analysis.

Chris M Frampton: Study design, data analysis, data interpretation, manuscript review editing.

Rod Jackson: reviewing and advising on the analytical approach; interpretation of the findings and commenting on drafts of the paper.

Katrina Poppe: data interpretation, methodological input, writing review and editing.

Carlos Arturo Camargo Jr: - funding acquisition, investigation, writing (review & editing).

Vicky A Cameron.: principal investigator for HVOLS cohort; review of manuscript.

Robert Scragg: Conceptualisation, funding acquisition, project administration, resources, supervision, writing - review & editing.

I have access to the data and can verify the underlying data reported in the manuscript.

A Mark Richards: conception, design, raised funding for assays, oversight of statistical analyses, wrote manuscript, edited, revised, corresponding author.

**Data sharing statement**

Data, including de-identified individual participant data with data dictionary, can potentially be made available to others subject to approval of a written proposal by both ViDA and HVOLS investigators’ steering groups. Contact on potential data sharing can be made via the corresponding author at mark.richards@cdhb.health.nz.

**Declaration of interests**

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**Supplementary materials**

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