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FULL RESEARCH PAPER
CVD risk factors

Sex-specific added value of cardiac biomarkers for 10-year cardiovascular risk prediction

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Aims To evaluate the sex-specific predictive value of N-terminal pro B-type natriuretic peptide (NT-proBNP), high sensitivity cardiac troponin T (hs-cTnT) and creatine kinase myocardial band (CK-MB) for 10-year risk prediction of coronary heart disease (CHD), stroke, heart failure (HF) and composite outcomes.

Methods Five-thousand four-hundred thirty individuals (mean age 68.6 years, 59.9% women) from the Rotterdam Study, with biomarker measurements between 1997 and 2001, were included. Participants were followed until 2015. We fitted 'basic' models using traditional cardiovascular risk factors. Improvements in c-statistics and net reclassification improvement (NRI) for events and non-events were calculated.

Results During a median follow-up of 14 years, 747 (13.8%), 563 (10.4%), and 664 (12.2%) participants were diagnosed with CHD, stroke, and HF, respectively. NT-proBNP improved the discriminative performance of the 'basic' model for all endpoints (c-statistic improvements ranging from 0.007 to 0.050) and provided significant event-NRI for HF (14.3% in women; 10.7% in men) and for stroke in men (9.3%). The addition of hs-cTnT increased c-statistic for CHD in women by 0.029 (95% CI, 0.011–0.047) and for HF in men by 0.034 (95% CI, 0.014–0.053), and provided significant event-NRI for CHD (10.3%) and HF (7.8%) in women, and for stroke (8.4%) in men. The added predictive value of CK-MB was limited.

Conclusion NT-proBNP and hs-cTnT provided added predictive value for various cardiovascular outcomes above traditional risk factors. Sex differences were observed in the predictive performance of these biomarkers.

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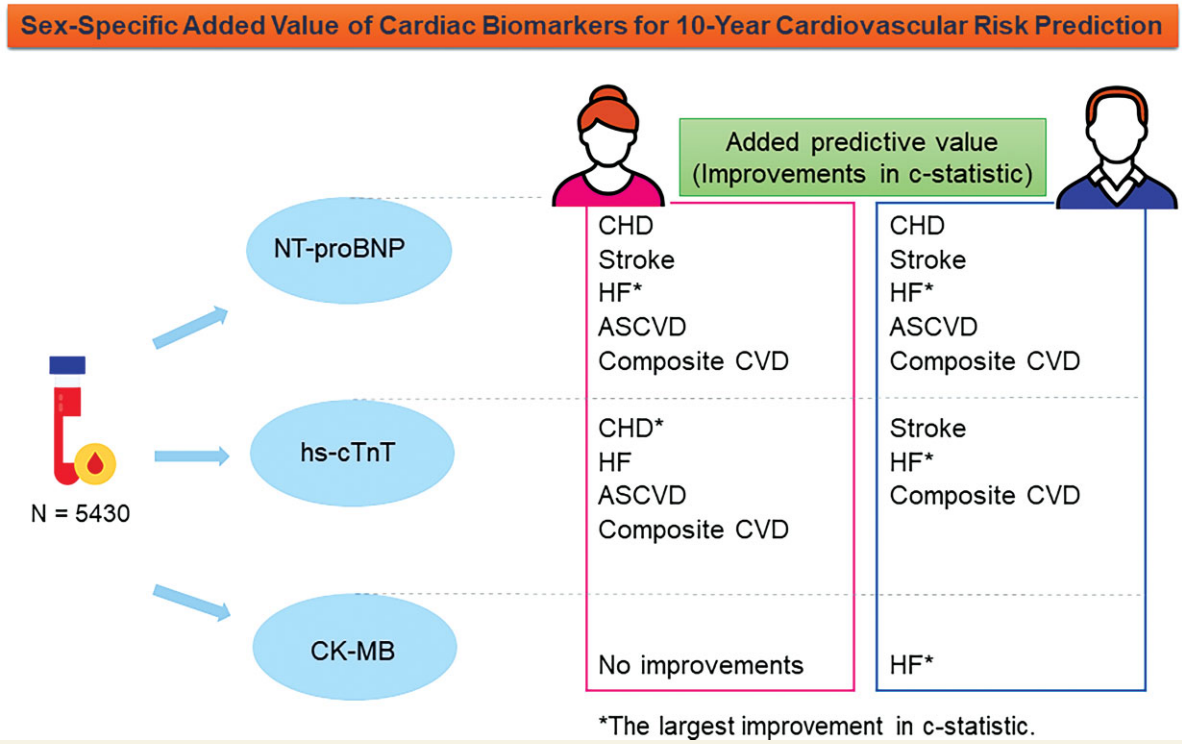
Graphical Abstract



NT-proBNP and hs-cTnT, but not CK-MB, showed add predictive value for various cardiovascular outcomes above traditional risk factors, especially for HF risk prediction. Sex differences were observed in the predictive performance of these cardiac biomarkers.

Keywords NT-proBNP • Troponin T • Creatine kinase myocardial band • Risk prediction • Cardiovascular disease • Sex differences

Introduction

Accurate cardiovascular risk assessment for early identification of individuals that would qualify for intensified lifestyle and medical interventions, is endorsed by major guidelines as a cornerstone of primary prevention of cardiovascular disease (CVD).[1,2] To reduce residual cardiovascular risk and further improve the accuracy of cardiovascular risk prediction and classifications, the addition of new biomarkers to the risk prediction models has increasingly gained interest.[3]

Plasma cardiac biomarkers integrate signals from different pathophysiological pathways underlying cardiac and vascular pathology. N-terminal pro B-type natriuretic peptide (NT-proBNP) is a well-established biomarker of heart failure (HF) and cardiac dysfunction, and its release is primarily stimulated by myocyte stretch in response to increased ventricular blood volume or filling pressure.[4] High sensitivity cardiac troponin T (hs-cTnT) and creatine kinase myocardial band (CK-MB) are both specific biomarkers of myocardial injury.[5] In otherwise healthy individuals, the concentrations of these three biomarkers reflect accumulated subclinical cardiovascular damage and are associated with future cardiovascular events.[5–8] Although emerging evidence support the role of NT-proBNP as a promising predictive biomarker for cardiovascular events in general population,[9–14] most previous studies explored specific cardiovascular outcomes, with HF accounting for the majority. However, contemporary prevention guidelines have a strong focus on global atherosclerotic cardiovascular risk, mostly encompassing a composite of coronary heart disease (CHD) and ischemic stroke risk.[2] Comparisons of the predictive ability of these cardiac biomarkers on individual and composite cardiovascular outcomes are scarce. Moreover, despite differences in pathophysiology of cardiovascular diseases between women and men,[15] sex differences in the predictive value of these cardiac markers in cardiovascular risk prediction have not been thoroughly explored.

Using data from the prospective population-based Rotterdam Study, we determined the predictive performance of NT-proBNP, hs-cTnT, and CK-MB, above traditional cardiovascular risk factors for predicting CHD, stroke, HF and composite outcomes among women and men without established CVD.

Methods

Study population

The current study was embedded within the Rotterdam Study (RS), an ongoing prospective population-based cohort among inhabitants from the suburb Ommoord in Rotterdam, the Netherlands. The initial cohort started from 1990 (RS-I) and included participants aged 55 years and over. RS cohort has undergone three extensions. In 2000, RS cohort was expanded with the second sub-cohort (RS-II) consisting of

participants who aged 55 years and over, or moved into the study area. In 2006, participants aged 45–54 years were included in extended subcohort (RS-III). In 2016, the recruitment of another extension (RS-IV) started the targeted population aged 40 years and over. The participants were examined at baseline and subsequent follow-up examinations that have been taking place every 3 to 4 years. Detailed rationale and design of the cohort have been described previously.[16]

This study was conducted using data from the third visit of the original cohort (RS-I-3: 1997–1999, $n = 4797$), and the first visit of the second cohort (RS-II-1: 2000–2001, $n = 3011$). Of these participants, 6693 visited the study center (RS-I-3: $n = 4063$ and RS-II-1: $n = 2630$). Participants with a history of CHD, stroke, or HF ($n = 955$) or incomplete information on the history of these diseases ($n = 159$) were excluded. Of the 5579 participants, 5430 participants had NT-proBNP measurement, and of these 5430, 4244 for hs-cTnT and 4423 for CK-MB, 4239 participants had both hs-TnT and CK-MB measurements. Missing cardiac biomarkers' measurements were considered random. The final sample for analyses included 5430 participants for NT-proBNP analyses, 4239 participants (as a nested dataset of NT-proBNP) for hs-cTnT and CK-MB analyses.

The RS has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare, and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The RS has been entered into the Netherlands National Trial Register (www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (www.who.int/ictrp/network/primary/en/) under shared catalog number NTR6831. All participants provided written informed consent to participate in the study and to have their information obtained information from their treating physicians.

Assessment of cardiac biomarkers

Serum NT-proBNP was measured using a commercially available electrochemiluminescence immunoassay (Elecsys proBNP, F Hoffman-La Roche Ltd) on an Elecsys 2010 analyzer, which measures concentrations ranging from 0.6 to 4130 pmol/L. hs-cTnT and CK-MB (measured by mass assay) were both measured using an electrochemiluminescence immunoassay on a cobas e 801 immunoassay analyzer, which measures hs-cTnT concentrations ranging from 3 to 10 000 ng/L and CK-MB concentrations ranging from 0.3 to 300 μg/L.

Outcome measures

The RS participants are monitored continuously for incident cardiovascular events and mortality through automated linkage with the digital records from general practitioner. The clinical events included incident CHD, stroke, and HF, and two composite endpoints which were atherosclerotic CVD (ASCVD, the occurrence of either CHD or stroke), and global CVD (the occurrence of either CHD, stroke, or HF). Detailed information regarding event identification and adjudication have been described previously.[17] In short, incident CHD was defined as fatal or nonfatal myocardial infarction or death from CHD.[17] The definition of stroke was according to the World Health Organization criteria as a syndrome of rapidly developing symptoms of focal or global cerebral dysfunction lasting 24 h or longer or leading to death, with no other apparent cause than of vascular origin.[18] Incident HF was defined as a combination of the presence of typical symptoms or signs of HF based on the criteria of the European Society of Cardiology and confirmed by a medical specialist.[17] In current study, participants were followed for the occurrence of the cardiovascular outcomes, death, or end of follow-up (January 2015), whichever came first.

Assessment of cardiovascular risk factors

Blood pressure was measured using a random-zero sphygmomanometer on the right arm twice and the average of the two measurements was used. Diabetes mellitus was defined as a fasting glucose level ≥7 mmol/L (125 mg/dL) or non-fasting glucose level ≥11.1 mmol/L (200 mg/dL), and/or the use of glucose lowering medication. Serum total cholesterol and HDL cholesterol were assessed using comparable enzymatic procedures. Estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration formula.[19] Trained nonmedical interviewers used a standardized questionnaire to obtain information on medical history, smoking status, and medication use. Smoking status was defined as current vs. non-current smoking.

Statistical analysis

Baseline characteristics of the study population were presented as mean (standard deviation, SD) for normally distributed variables, median [interquartile range (IQR)] for skewed variables, and number (percentage) for categorical variables. Measures of NT-proBNP, hs-cTnT and CK-MB were log-transformed to approximate normal distribution. We used student's t-test for continuous data and χ^2-test for categorical data to compare baseline characteristics between women and men.

Cox proportional hazards models were constructed to assess associations of NT-proBNP, hs-cTnT and CK-MB with incident cardiovascular events. Interaction effects between sex and these cardiac biomarkers were tested. The non-linearity of the associations for log-transformed NT-proBNP, hs-cTnT, and CK-MB was tested using likelihood ratio tests and restricted cubic spline (five knots at the 5th, 35th, 50th, 65th, and 95th percentiles). Taking Akaike's Information Criteria and Bayesian Information Criteria into consideration, models including the non-linear terms did not show better performance. Therefore, to avoid overfitting, we treated the associations between markers and outcomes as linear in the analyses.

To evaluate the improvement in risk prediction, we fitted a 'basic' model based on the traditional cardiovascular risk factors included in the pooled cohort equations (PCEs)[1]: age, systolic blood pressure (SBP), treatment for hypertension, total and HDL cholesterol levels, current smoking, and diabetes. We then extended the 'basic' model with single addition of NT-proBNP, hs-cTnT, or CK-MB. The discriminative performance of the models was assessed using the c-statistic. Optimism-corrected c-statistics of 'basic' and 'extended' models were calculated based on 500 bootstrap replications. The 95% confidence intervals (95% CIs) for the differences between the c-statistic of the 'basic' model and 'extended' models (delta c-statistic) were calculated based on the bootstrap samples.

Further, risk categories were created using cut-offs defined by 2019 American College of Cardiology/American Heart Association (ACC/AHA) Guideline on the Primary Prevention of Cardiovascular Disease,[1] classifying individuals into low risk (<5%), borderline risk (5–7.5%), intermediate risk (7.5–20%), and high risk (>20%). We then computed the net reclassification improvement (NRI) for events and non-events after the extension of the 'basic' model with each cardiac biomarker (i.e. 'extended' models).[20] The 95% CIs for NRIs were calculated according to the method proposed by Pencina et al.[21]

In sensitivity analyses, we conducted for risk prediction models by excluding participants with lipid-lowering medication use at baseline. We also repeated all analyses after adding eGFR to the base model. Besides, Fine-Gray subdistribution hazard models were constructed to evaluate the associations between these biomarkers and cardiovascular outcomes,[22] considering mortality as a competing event. The maximum missing information on cardiovascular risk factors was up to 4.2% of the

Table 1 Baseline characteristics of the study population (*N* = 5430)

Variables	Women (*N* = 3255)	Men (*N* = 2175)	*P* value
Age, years	69.2 ± 8.4	67.7 ± 7.6	<0.001
Body mass index, kg/m^2	27.3 ± 4.4	26.5 ± 3.2	<0.001
Diabetes mellitus, *n* (%)	359 (11.1)	286 (13.3)	0.022
Systolic blood pressure, mm Hg	143 ± 21	144 ± 21	0.016
Hypertension treatment, *n* (%)	1019 (32.8)	546 (26.1)	<0.001
Total cholesterol, mmol/L	6.0 ± 0.9	5.6 ± 0.9	<0.001
HDL cholesterol, mmol/L	1.5 ± 0.4	1.3 ± 0.3	<0.001
Lipid-lowering medication, *n* (%)	337 (10.7)	174 (8.2)	0.003
Current smoking, *n* (%)	556 (17.3)	531 (24.6)	<0.001
NT-proBNP, pmol/L	10 (6–18)	7 (4–14)	<0.001
hs-cTnT, ng/L[a]	6 (4–9)	9 (6–13)	<0.001
CK-MB, μg/L[a]	2.0 (1.6–2.8)	2.4 (1.8–3.3)	<0.001

Data presented as mean ± SD, median (IQR), or number (percentage).
[a]Median (IQR) calculated among 4239 participants with available hs-cTnT and CK-MB data. The presented *P*-value is for the differences in baseline characteristics between women and men.
CK-MB, creatine kinase- myocardial band; NT-proBNP, N-terminal fragment of prohormone B-type natriuretic peptide; hs-cTnT, high-sensitivity cardiac troponin T.

participants. Missing values in variables were imputed using multiple imputation method (Mice package in R). In the Cox regression analyses, five imputed datasets were generated and the summarized estimates were calculated based on Rubin's rule using the pool function in Mice.[23] A 2-sided *P*-value was considered significant at $P < 0.05$. All statistical analyses were performed with the use of R version 4.0.3 (https://www.r-project.org).

Results

Baseline characteristics

The study included 5430 participants with a mean (± SD) age of 68.6 ± 8.1 years, 59.9% of whom were women. *Table 1* shows baseline characteristics of the study population. Briefly, women had higher BMI, HDL and total cholesterol levels, and a higher proportion of lipid-lowering medication. Men had a larger proportion of diabetes mellitus and were more frequently current smokers. Although men had higher SBP levels, they had a lower proportion of hypertension treatment, compared to women.

The three cardiac biomarkers had right-skewed distributions. Median (IQR) baseline levels of NT-proBNP were 10 (6–18) pmol/L in women, 7 (4–14) pmol/L in men, and 9 (5–17) pmol/L in total population. Median levels of hs-cTnT were 6 (4–9) ng/L in women, 9 (6–13) ng/L in men, and 7 (5–11) ng/L in total population. Median levels of CK-MB were 2.0 (1.6–2.8) μg/L in women, 2.4 (1.8–3.3) μg/L in men, and 2.2 (1.6–3.0) μg/L in total population. Supplementary material online, *Table S1* shows baseline characteristics of 4239 participants for the hs-cTnT and CK-MB analyses.

Follow-up

The median follow-up was 13.6, 13.9, 13.6 years for CHD, stroke and HF respectively. During follow-up, a total of 747 (13.8%) participants were diagnosed with CHD, 563 (10.4%) participants had a stroke, and 664 (12.2%) participants were diagnosed with HF. A total of 1225 (22.6%) participants were diagnosed with ASCVD, and 1597 (29.4%) participants were diagnosed with global CVD.

Association of cardiac biomarkers with incident cardiovascular events

Table 2 displays adjusted hazard ratios (HRs) of incident cardiovascular events for the continuous cardiac biomarkers. After adjusting for cardiovascular risk factors including age, SBP, hypertension treatment, total and HDL cholesterol levels, lipid-lowering medication, smoking status, and diabetes status, higher levels of NT-proBNP and hs-cTnT were significantly associated with higher risk for all cardiovascular events in both sexes. For the separate cardiovascular endpoints, higher levels of CK-MB were only associated with risk of HF in men (HR 1.39; 95% CI, 1.21–1.59). We observed significant interaction between sex and NT-proBNP for ASCVD outcome ($P = 0.030$) and global CVD outcome ($P = 0.001$); significant interaction between sex and hs-cTnT for CHD ($P = 0.013$) and ASCVD outcome ($P = 0.025$); and significant interaction between sex and CK-MB for HF outcome ($P = 0.04$). Supplementary material online, *Table S2* shows results of subdistribution hazard models for cardiovascular events, treating mortality as a competing event. Results for the associations between cardiac biomarkers and cardiovascular outcomes were overall in line with the ones obtained from Cox proportional hazards models.

Discriminative performance of cardiac biomarkers for 10-year cardiovascular risk prediction

Improvements in c-statistic for 10-year risk prediction for incident cardiovascular events after adding cardiac biomarkers are shown in *Figure 1*. For all outcomes, the optimism-corrected c-statistic significantly improved after extending the 'basic' model with adding NT-proBNP. The largest increase in c-statistic of the 'extended' models was for HF, which was 0.027 in women and 0.050 in men.

Table 2 Association of cardiac biomarkers with cardiovascular outcomes

Outcomes	Women			Men		
	Event/*N*	HR (95% CI)	*P*-value	Event/*N*	HR (95% CI)	*P* value
CHD						
NT-proBNP	351/3255	1.29 (1.15–1.46)	<0.001	396/2175	1.33 (1.19–1.49)	<0.001
hs-cTnT	243/2505	1.46 (1.28–1.67)	<0.001	311/1734	1.24 (1.09–1.41)	0.001
CK-MB	243/2505	1.11 (0.98–1.26)	0.089	311/1734	1.07 (0.95–1.20)	0.283
Stroke						
NT-proBNP	336/3255	1.40 (1.25–1.58)	<0.001	227/2175	1.38 (1.19–1.61)	<0.001
hs-cTnT	245/2505	1.36 (1.19–1.57)	<0.001	167/1734	1.32 (1.11–1.56)	0.002
CK-MB	245/2505	1.09 (0.96–1.24)	0.166	167/1734	1.11 (0.95–1.31)	0.189
HF						
NT-proBNP	392/3255	1.89 (1.69–2.10)	<0.001	272/2175	1.91 (1.68–2.17)	<0.001
hs-cTnT	257/2505	1.50 (1.32–1.70)	<0.001	201/1734	1.69 (1.46–1.95)	<0.001
CK-MB	257/2505	1.12 (0.99–1.27)	0.060	201/1734	1.39 (1.21–1.59)	<0.001
ASCVD[a]						
NT-proBNP	640/3255	1.35 (1.24–1.48)	<0.001	585/2175	1.37 (1.25–1.50)	<0.001
hs-cTnT	455/2505	1.44 (1.30–1.59)	<0.001	452/1734	1.29 (1.16–1.43)	<0.001
CK-MB	455/2505	1.12 (1.02–1.23)	0.015	452/1734	1.09 (0.99–1.20)	0.083
Global CVD[b]						
NT-proBNP	875/3255	1.56 (1.45–1.69)	<0.001	722/2175	1.48 (1.36–1.61)	<0.001
hs-cTnT	612/2505	1.44 (1.32–1.57)	<0.001	552/1734	1.38 (1.25–1.51)	<0.001
CK-MB	612/2505	1.11 (1.03–1.21)	0.009	552/1734	1.17 (1.08–1.28)	<0.001

All models are adjusted for age, current smoking, total cholesterol, HDL cholesterol, systolic blood pressure, hypertension treatment, diabetes.
Hazard ratios were calculated per 1 standard deviation increase of log-transformed biomarkers.
[a]ASCVD comprises coronary heart disease and stroke,
[b]Global CVD comprises coronary heart disease, stroke, and heart failure.
ASCVD, atherosclerotic cardiovascular disease; CHD, coronary heart disease; CK-MB, creatine kinase-myocardial band; HF, heart failure; global CVD, global cardiovascular disease; NT-proBNP, N-terminal fragment of prohormone B-type natriuretic peptide; hs-cTnT, high-sensitivity cardiac troponin T.

After addition of hs-cTnT to the 'basic' model, the c-statistic significantly improved for CHD (delta c-statistic, 0.029; 95% CI: 0.0011–0.047), HF (delta c-statistic, 0.017; 95% CI: 0.007–0.027), ASCVD (delta c-statistic, 0.020; 95% CI: 0.009–0.030), and global CVD (delta c-statistic, 0.018; 95% CI: 0.009 to 0.026) in women; and for stroke (delta c-statistic, 0.013; 95% CI: 0.001–0.026), HF (delta c-statistic, 0.034; 95% CI: 0.014–0.053) and global CVD (delta c-statistic, 0.012; 95% CI: 0.003–0.020) in men. In contrast, the addition of CK-MB only significantly improved c-statistic for HF in men (delta c-statistic, 0.015; 95% CI: 0.001–0.029). Supplementary material online, *Table S3* shows numerical data regarding the c-statistic for the 'basic' model and the 'extended' models of the three cardiac biomarkers.

Categorical NRI was used to assess the ability of the 'extended' models with biomarkers to correctly reclassify participants over the 'basic' model. As displayed in *Table 3*, the addition of NT-proBNP led to both significant event NRI (14.3; 95% CI: 7.8–20.8%) and non-event NRI (2.7; 95% CI: 0.9–4.4%) for HF in women. NT-proBNP also provided significant event NRI for stroke (9.3; 95% CI: 3.1–15.4%) and HF (10.7; 95% CI: 2.8–18.5%) in men. The addition of hs-cTnT greatly improved event NRI for CHD in women (10.3; 95% CI: 3.7–16.9%) and stroke in men (8.4; 95% CI: 1.9–14.9%), but the non-event NRI was modest. Addition of CK-MB did not result in significant improvements in risk reclassification. Supplementary material online, *Figures S1–S5* show event and non-event risk reclassifications for all outcomes with addition of the cardiac biomarkers.

We also evaluated the collective discriminative performance of NT-proBNP and hs-cTnT (see Supplementary material online, *Table S4*). We did not include CK-MB because of its limited performance in improving c-statistic and NRI. Compared to an extended model with the single addition of NT-proBNP, simultaneous addition of both NT-proBNP and hs-cTnT only slightly improved predictions. In Supplementary material online, *Table S5* and *S6*, we provided equation parameters of NT-proBNP, and hs-TnT extended risk prediction models, and case examples for calculation of 10-year risk.

In sensitivity analyses, the predictive performance of cardiac biomarkers did not change materially after excluding participants using lipid-lowering medication. The result did not change after adding eGFR to the base model (data not shown).

Discussion

In this study, addition of NT-proBNP or hs-cTnT to a model including traditional cardiovascular risk factors significantly improved model performance for 10-year risk predictions for various cardiovascular outcomes. Addition of NT-proBNP provided relevant

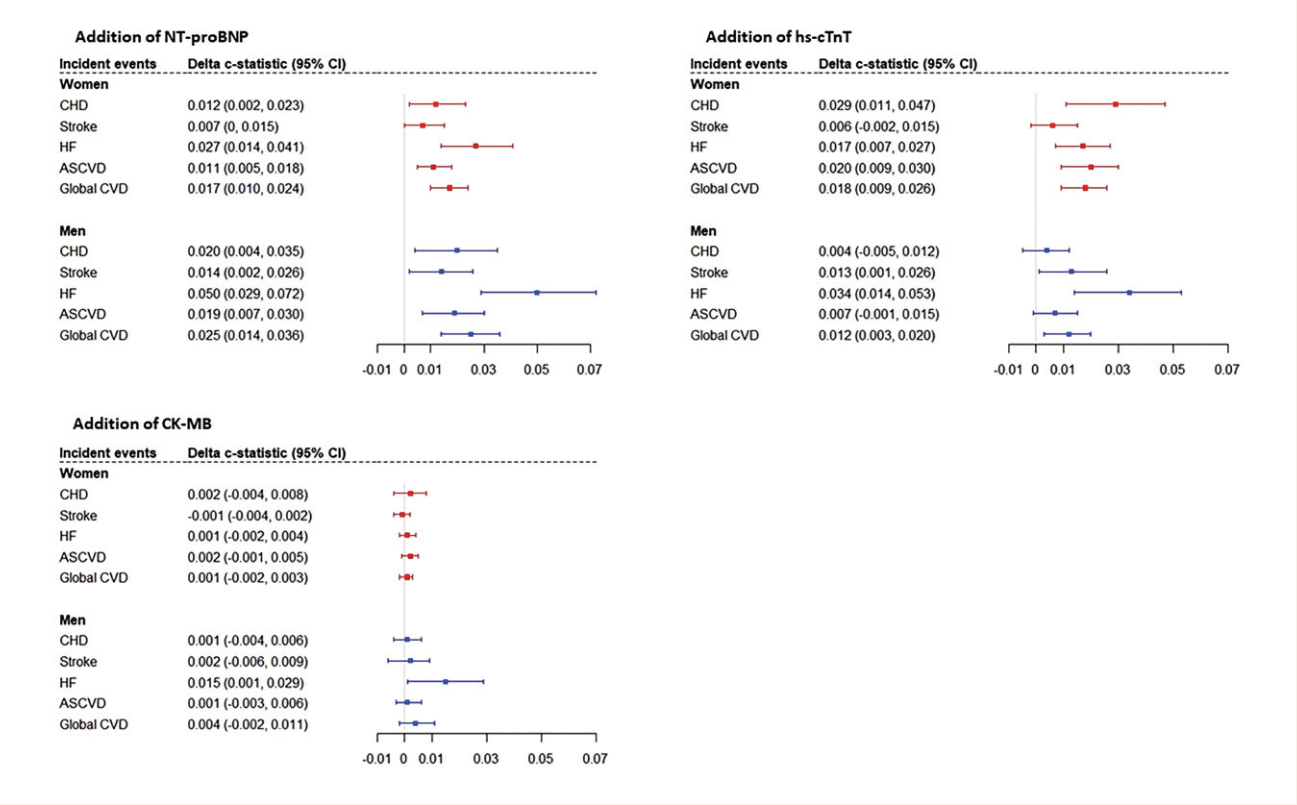


Figure 1 Improvements in c-statistic for 10-year risk prediction for incident cardiovascular events after adding cardiac biomarkers. Delta c-statistic denotes difference in the c-statistic between extended models (after addition of cardiac biomarkers) compared to the 'basic' model. Atherosclerotic cardiovascular disease comprises coronary heart disease and stroke; global cardiovascular disease comprises coronary heart disease, stroke and heart failure. CHD, coronary heart disease; CK-MB, creatine kinase-myocardial band; HF, heart failure; hs-cTnT, high-sensitivity cardiac troponin T; NT-proBNP = N-terminal fragment of prohormone B-type natriuretic peptide.

Table 3 Improvements in risk reclassification for cardiovascular events after adding cardiac biomarkers

Incident events	Addition of NT-proBNP		Addition of hs-cTnT		Addition of CK-MB	
	Event NRI	Non-event NRI	Event NRI	Non-event NRI	Event NRI	Non-event NRI
Women						
CHD	4.8 (−0.1, 9.8)	−0.1 (−1.5, 1.2)	10.3 (3.7, 16.9)	0.3 (−1.4, 1.9)	1.6 (−2.9, 6.2)	0.8 (−0.2, 1.8)
Stroke	3.9 (−1.7, 9.5)	−0.1 (−1.7, 1.5)	3.3 (−2.5, 9.0)	−1.5 (−3.1, 0.2)	−0.4 (−4.1, 3.3)	0.6 (−0.4, 1.6)
HF	14.3 (7.8, 20.8)	2.7 (0.9, 4.4)	7.8 (1.3, 14.3)	−0.2 (−1.7, 1.4)	3.9 (0.5, 7.3)	0.3 (−0.7, 1.2)
ASCVD[a]	2.5 (−1.4, 5.7)	3.1 (1.3, 4.8)	4.6 (0.6, 8.7)	2.9 (0.8, 5.0)	−1.3 (−4.2, 1.5)	0.9 (−0.5, 2.3)
Global CVD[b]	1.6 (−1.6, 4.8)	6.5 (4.3, 8.7)	0.5 (−2.9, 3.8)	5.1 (2.9, 7.3)	−1.0 (−2.7, 0.8)	1.6 (0.2, 2.9)
Men						
CHD	2.8 (−1.7, 7.2)	0.4 (−1.7, 2.6)	3.2 (−1.0, 7.5)	1.7 (−0.4, 3.7)	0.0 (−2.5, 2.5)	1.8 (0.5, 3.1)
Stroke	9.3 (3.1, 15.4)	−2.9 (−4.9, -0.9)	8.4 (1.9, 14.9)	−0.9 (−3.1, 1.3)	3.0 (−1.5, 7.5)	1.0 (−0.6, 2.6)
HF	10.7 (2.8, 18.5)	0.6 (−1.8, 3.0)	6.0 (−1.8, 13.7)	0.8 (−1.4, 3.1)	0.0 (−6.3, 6.3)	3.7 (1.6, 5.7)
ASCVD[a]	2.4 (−0.9, 5.7)	1.6 (−0.4, 3.6)	0.4 (−2.7, 3.6)	0.3 (−1.7, 2.4)	−0.2 (−2.1, 1.7)	0.9 (−0.4, 2.1)
Global CVD[b]	2.9 (0.1, 5.8)	4.5 (2.3, 6.8)	2.2 (−0.8, 5.2)	1.8 (−0.4, 3.9)	0.4 (−2.1, 2.8)	2.2 (0.5, 3.9)

NRI (%) was calculated using cut points for 10-year risk set at 5%, 7.5%, and 20% for cardiovascular events.
[a]ASCVD comprises coronary heart disease and stroke.
[b]Global CVD comprises coronary heart disease, stroke, and heart failure.
ASCVD, atherosclerotic cardiovascular disease; CHD, coronary heart disease; CK-MB = creatine kinase-myocardial band; HF, heart failure; global CVD = global cardiovascular disease; hs-cTnT = high-sensitivity cardiac troponin T; NRI = net reclassification improvement; NT-proBNP, N-terminal fragment of prohormone B-type natriuretic peptide.

improvements in risk reclassifications of cardiovascular outcomes. hs-cTnT mainly improved risk reclassifications for CHD and HF in women and for stroke in men. The predictive performance of CK-MB for improving cardiovascular risk predictions was limited. Moreover, sex differences were observed in the magnitude of these risk prediction improvements.

Major guidelines for primary prevention of CVD recommend use of risk scoring algorithms as the basis for clinical decision making.[2,24] The well-established cardiovascular risk prediction equations only include traditional cardiovascular risk factors as predictors, and cardiac biomarkers are rarely measured for this purpose in clinical practice. Using contemporary clinically meaningful risk thresholds recommended in the most recent ACC/AHA guideline,[1] we assessed the predictive properties of three cardiac biomarkers for broader cardiovascular outcomes.

NT-proBNP has received considerable attention as biomarker of cardiac dysfunction and HF.[5,25] In addition to its use in clinical practice for HF diagnosis, the concentration of NT-proBNP has been found to be a promising predictor of long-term cardiovascular events.[26] In line with previous studies,[11,13,26] our study confirmed the predictive value of NT-proBNP for risk discrimination for various CVD outcomes in both men and women.

In our study, hs-cTnT provided significant improvement in model discrimination for CHD and ASCVD prediction in women, stroke prediction in men, HF and global CVD prediction in both sexes. Ischemia, inflammation, oxidative stress, and neurohormonal activation can induce cardiomyocyte injury resulting in troponin release.[27] It has been well-established that cardiac structural and functional abnormalities,[28] and coronary atherosclerosis[29] lead to stable modest increases in troponin concentrations in the circulation. However, evidence on the predictive value of hs-cTnT for cardiovascular risk is limited and inconsistent. Willeit *et al*[8] reported that the addition of hs-cTnT improved the c-statistic for the prediction of fatal CVD, whereas no significant improvements were observed for the overall CVD outcome. Saunders et.al[7] and McEvoy et.al[30] demonstrated hs-TnT significantly improved CHD and HF risk prediction in primary prevention populations. Recently, the Atherosclerosis Risk in Communities study investigators[31] have reported a larger c-statistic increase by adding hs-cTnT to PCE variables-based model for global CVD risk prediction compared to our study. The ARIC study determined the predictive properties of hs-cTnT for short-term CVD risk prediction based on 4 years of follow-up, while the median follow-up in our study was about 14 years, moreover the RS population was on average 7 years younger. It has been well-established that risk prediction models (and by extension added biomarkers) generally perform better at predicting short-term risk and in younger individuals. Besides, it is worth noting that both NT-proBNP and hs-cTnT concentrations are related to renal function.[32,33] However, in the traditional risk prediction models, including the PCE, kidney function parameters are not considered. We further incorporated renal function measure into the base model in sensitivity analysis, and the predictive performance of the biomarkers did not change.

With the adoption of troponins, the use of CK-MB in diagnosis of myocardial infarction has markedly diminished over time. CK-MB is less cardiac-specific than troponins. Levels of CK-MB can be elevated by pathologic processes including skeletal muscle injury and renal failure.[34] The clinical utility and cost-effectiveness of CK-MB have been questioned.[34,35] In our study, we observed that increases in CK-MB was weakly associated with incident HF and combined CVD outcomes. Moreover, CK-MB only provided minor incremental predictive properties for HF risk prediction in men.

To be clinically applicable, the new cardiovascular biomarkers should lead to clinically relevant risk reclassifications. Good risk prediction models can adequately distinguish individuals who are expected to benefit most from preventive therapies.[13] Our study showed that the clinical applicability of NT-proBNP for HF risk reclassification was promising. Hs-cTnT showed significant improvements in CHD and HF risk reclassification in women and stroke risk reclassification in men. Previous studies reported NT-proBNP provided significant improvements in CHD, HF, and stroke risk reclassifications.[10,11,26] One meta-analysis reported low overall categorical NRIs of 0.027 for ASCVD and 0.028 for global CVD for NT-proBNP.[26] For hs-cTnT, it has been found that it could improve HF and global CVD reclassifications.[31,36] This discrepancy could result from employing the most recent 2019 ACC/AHA guideline risk thresholds in our study, which is different from most previous studies, as categorical NRI calculation depends on placement of risk thresholds and the total number of risk categories.[20,21]

To date, few studies have explored sex differences in the added predictive value of NT-proBNP and hs-cTnT. Women are known to have higher NT-proBNP and lower hs-cTnT concentrations than men.[37] This is also an observation made in our study. While still far from being comprehensively understood, several mechanisms may contribute to the differences in concentrations between women and men, including body composition, cardiac mass, and sex hormones.[37,38] Notably, we observed sex differences in the associations and predictive values of these cardiac biomarkers with risk of cardiovascular events. To add, the model adjustments used in our study, based on the PCE predictors, did not include information on body composition, sex hormones, and cardiac morphology that may confound comparisons of cardiac biomarkers between sexes.[37]

The strengths of our study include availability of data on several cardiac biomarkers among community-dwelling participants, a well-characterized cohort, and meticulous adjudication of various cardiovascular endpoints. There are also some limitations. First, the study population comprised older white individuals (98% white). Therefore, our results might not be generalizable to younger populations and other ancestries. Second, we select predictors derived from PCE variables, which have previously been shown to be promising predictors of cardiovascular events in the general population. It is possible that other biomarkers that were not included would have provided additional predictive information.

Conclusions

In our population-based study, NT-proBNP and hs-cTnT improved model performance for 10-year risk predictions for various cardiovascular outcomes, beyond traditional risk factors, for both women and men. Both biomarkers provided relevant improvements in risk reclassifications for HF and stroke. Sex differences were observed in the predictive performance of these cardiac biomarkers.

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Authors' contributions

F.Z. and M.K. contributed to the conception or design of the work. F.Z., B.A., M.J.G.L., E.B., and M.K. contributed to data acquisition, and data interpretation. F.Z. did the statistical analysis and drafted the manuscript. F.Z., B.A., M.J.G.L., E.A., M.A.K., E.B., M.K.I., and M.K. critically revised manuscript. All gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

Supplementary material

Supplementary material is available at *European Journal of Preventive Cardiology*.

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Conflict of interest: None declared.

Data availability

Data generated by the authors or analyzed during the study are available upon request. Requests should be directed towards the management team of the Rotterdam Study (secretariat.epi@erasmusmc.nl), which has a protocol for approving data requests. Because of restrictions based on privacy regulations and informed consent of the participants, data cannot be made freely available in a public repository.

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