Association of Repeatedly Measured High-Sensitivity-Assayed Troponin I with Cardiovascular Disease Events in a General Population from the MORGAM/BiomarCaRE Study

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Short title: Prognostic Importance of Troponin Changes for General Population

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Abbreviations list:

hs-cTnI high sensitivity troponin I, cTnT contemporary troponin T

CVD Cardiovascular disease

MI Multiple Imputation

JM Joint model

RF Risk factors

R1, round 1, R2 round 2, R3 round 3

HR hazard ratio

LoD limit of detection

NRI Net reclassification Index

GDF15 Growth Differentiation Factor 15

NTProBNP N-terminal pro Brain natriuretic peptide

Abstract

Background: Levels of high sensitivity troponin I (hs-cTnI) reflect myocardial stress. The role of hs-cTnI in predicting long term changes in the risk of cardiovascular disease (CVD) in general populations is not clearly defined.

Methods: We investigate whether the change in three repeated measures of hs-cTnI collected five years apart in a prospective Danish study (3875 participants, initially aged 30-60, 51% female, disease free at baseline), improves 10-year prediction of incident CVD compared to using a single most recent hs-cTnI measurement. The change process was modelled using a joint (longitudinal and survival) model and compared to a Cox model using a single hs-cTnI measure adjusted for classic CVD risk factors, and evaluated using discrimination statistics.

Results: Median hs-cTnI levels changed from 2.6ng/L to 3.4ng/L over 10 years. The change in hs-cTnI predicts 10-year risk of CVD (581 events); the joint model gave a HR 1.31 per interquartile difference in hs-cTnI (95% C.I. 1.15, 1.48) after adjustment for CVD risk factors. However, the joint model performed only marginally better (c-index improvement 0.0041, p=0.03) than using a single hs-cTnI measure (c-index improvement 0.0052, p=0.04) for prediction of CVD, which is compared to a model incorporating CVD risk factors without hs-cTnI (c-index 0.744).

Conclusions: The change in hs-cTnI in 5 year intervals better predicts risk of CVD in the general population, but the most recent measure of hs-cTnI, (at 10 years) is as effective in predicting CVD risk. This simplifies the use of hs-cTnI as a prognostic marker for primary prevention of CVD in the general population.

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Introduction

Cardiac troponin is a marker of necrosis and of myocardial infarction in emergency situations (1) but can also act as a long term biomarker in predicting risk of CVD, heart failure and death in the general population (2, 3). High sensitivity measured troponin I (hs-cTnI) can be detected in 80-90% of the general population (3, 4) and adds predictive information beyond established risk factors for fatal and non-fatal CVD in men and women across a wide age-range (2, 3, 5, 6). As low levels of troponin (below limits of conventional troponin assays) can be prognostically important (3), biological distributions and long term variation of troponin levels and their relationship with other risk factors across general populations need to be characterised. Longitudinal studies repeatedly measuring troponin can address this and estimate the prognostic importance of measuring changes for predicting risk of disease. The determinants of temporal increases in troponin (hs-cTnT) over 6 years in a general population (median 57 yrs) have recently been shown to include increasing age, male gender, hypertension, diabetes and obesity (7). Changes in troponin over 1 year were associated with increased risk of myocardial infarction in stable coronary heart disease patients (8). In healthy elderly populations (>65 years) changes in troponin over 3-5 years were associated with increased risk of heart failure, cardiovascular and all-cause mortality and atrial fibrillation (9, 10, 11, 12). However, incorporating this change led to minimal improvement in risk prediction (9, 10, 11). In a healthy general population (mean 56 years) changes in troponin over 6 years failed to improve prediction of coronary heart disease but improved heart failure risk (13). No studies have assessed the prognostic implications of changes in troponin levels in general populations over longer time scales.

Using a prospective population cohort initially aged 30-60, which collected hs-cTnI and other risk factors at three time points over 10 years with a further 16 years of follow up for incident CVD events, we determine 1) if change in hs-cTnI is associated with increased risk of CVD, and 2) whether prediction of CVD can be improved by modelling change in hs-cTnI. We develop prognostic models comparing the trend in hs-cTnI over 10 years to a single most recent hs-cTnI measure.

Methods

Study design

The MONICA 1 population cohort at the Research Centre for Prevention and Health (RCPH) represents 11 municipalities in Copenhagen, Denmark. Men and women aged 30, 40, 50 and 60 were randomly sampled from the national population register (14). They were examined in 1982-84 (Round 1), then re-examined in 1987-1988 (Round 2) and again in 1993-1994 (Round 3) (14) (Figure 1). Participants received a physical examination, a self-administered questionnaire, and a blood sample was drawn at each exam. Smoking status, blood pressure, body mass index and blood lipids were measured in a standardized way. Prevalent diabetes was defined as self-reported doctor-diagnosed diabetes, use of diabetes medication or diabetes history recorded in registry data at each round. The study was approved by ethics committees; participants consented to all examinations and follow-up of their medical records. At recruitment, pre-existing cardiovascular disease (myocardial infarction or stroke) was self-reported. The outcome was the first major cardiovascular event (including first fatal or non-fatal definite or possible myocardial infarction, coronary death or unclassifiable death, unstable angina, cardiac revascularisation, and

probable ischemic stroke). Follow up was achieved through linkage to the National Cause of Death Register and National Hospital Discharge Register until December 2009 with only 40 participants (1.05%)lost to follow-up (15, 16). Registry diagnoses have been validated against the MONICA criteria (17).

Biomarker measurements and adjustments

Serum was separated from blood, then stored at 4ºC (1-3 days) during transfer to the laboratory for storage at -20ºC and subsequently at -80 ºC. Storage times are summarized in Supplemental table 1. Biomarkers were measured in the MORGAM/BiomarCaRE laboratory. hs-cTnI levels were determined by the ARCHITECT STAT high sensitivity Troponin I immunoassay (Abbott Diagnostics, USA, ARCHITECT i2000SR). The limit of detection (LoD) was 1.9 ng/L, with values below this imputed (see below). The assay supported a 10% coefficient of variation at a concentration of 5.2 ng/L. Intra-assay and inter-assay coefficients of variation were 4.3% and 6.3%. Because of skewed distributions, a cubic root transformation was applied to hs-cTnI for consistency with previous research (2, 3).

Statistical Analysis

Missing data occurred across the three rounds either due to participants failing to attend examination, insufficient information on risk factors or insufficient serum for a biomarker test. However their vital status was followed up. Participation rates across the rounds are given in Supplemental table 2. Missing data for risk factors was minimal (<0.1%) for those attending exams. Missing data for biomarkers ranged from 0.15% to 8.8% and hs-cTnI missingness was from 4.8% at round 1, 8.8% at round 2

and 0.51% at round 3. Missing data was addressed through a multiple imputation (MI) model which captured the longitudinal trajectory of hs-cTnI and other variables. Imputed values were applied to the risk prediction analysis models. Individuals recruited at round 1 were included in the imputation model. The MI model for men and women combined included classic CVD risk factors, biomarkers, and case status at the start and end of the follow-up. Twenty imputed datasets were created using chained equations (18). Predictive mean matching was used for all variables, except hs-cTnI, where a normal linear regression model was used. When the missing values corresponded to values below the LoD the imputed values were drawn from a truncated normal distribution, otherwise the imputed values were drawn from a normal distribution. Time-to-event information was included in the imputation model (19) with further details in supplemental material. A sensitivity analysis was performed on a restricted dataset of those with hs-cTnI and risk factors measured at round 3, who may have had some missing measures prior to that round, which were imputed (see supplemental methods).

Prediction models

Prediction modelling is outlined in Figure 1. We develop prognostic models comparing the trend in hs-cTnI over 10 years to a single most recent hs-cTnI measure. We need to keep the measurement period (10 years of change) separate to the follow-up period as prognostic models cannot use 'future measures' (i.e. R2 or R3 in this case) as predictors, therefore a new baseline at 10 years becomes the starting point for predictions using a further 16 years of follow-up after this point.

A Cox proportional hazards model was constructed containing cardiovascular risk factors: sex, systolic blood pressure (SBP), HDL-cholesterol, nonHDL-cholesterol (difference between total and HDL cholesterol), prevalent diabetes, smoking status and body mass index (BMI) measured at round 3 (Model 1). The round 3 measurements were the baseline for the follow-up and age was used as the time-scale in the analysis. The follow-up extended up to 16 years later. To this model, hs-cTnI measured at round 3 was added (Model 2). Hazard ratios are reported per 0.4 times the cubic root of the hs-cTnI concentration in ng/L which corresponds roughly to the interquartile range in the cohort.

Model 2 is compared to models 3-5 which incorporate the history of hs-cTnI and the cardiovascular risk factors measured in rounds 1-3. Model 3 is similar to model 2 but uses the change in hs-cTnI from round 1 to 3 as the predictor instead of the level of hs-cTnI at round 3. Those developing CVD before Round 3 were excluded from analysis.

Model 4 combines analysis of the repeated measurements and time to cardiovascular event. It is a joint model (JM) of the association between the repeated measurements at rounds 1-3 and the risk of CVD (20). The model forms two parts, a multilevel model for longitudinal trend in hs-cTnI and a proportional hazards survival model for cardiovascular events (supplemental equation). The multilevel model includes a random intercept reflecting a different starting value of hs-cTnI for each participant and its relationship with other covariates, and a random slope for time from baseline. Sex, age at the first examination (measuring the cross sectional effect of age) and time from baseline (measuring the longitudinal effect of age) are fixed effects in this model. The survival model incorporates: sex, SBP, HDL-cholesterol,

nonHDL-cholesterol, prevalent diabetes, smoking status and BMI, updated at each round. hs-cTnI is included in the survival model via the estimate from the multilevel model. The JM was fitted to all follow-up data but the validation step involving Model 4 uses only the follow-up data after round 3 (see below).

We also fitted a Cox model with time-dependent covariates using the same covariates as Model 4 and using the same timespan and number of events (Model 5). All survival models used age as the time scale (21, 22). For the Cox models, those developing CVD before R3 were excluded. For the joint models information available after an individual developed CVD was not used. Prevalent cases of CVD at the first examination (2.18%, N=83) were excluded from the analyses.

Fitting and validation of the prediction models

Prediction models 1-5 were discriminated using the C-index (23) and net reclassification index (NRI) (24) using the 10 year probabilities of CVD derived from the models using round 3 as the baseline. Ten-fold cross validation was used to adjust the risk estimates for over optimism in assessing model performance on the same dataset where it was developed (25, 26). The analyses were carried out in both genders combined, and performed using R v3.1.1 (R Core Team (2013) (27). Our study conforms to TRIPOD guidelines for reporting prediction models (28).

Results

Participant characteristics

Participant characteristics for each examination round are given in Table 1 (imputed data) and Supplemental table 1 (complete case data). From round 1 (R1) to round 3 (R3), the prevalence of diabetes increased from 2.2% to 4.3%, smoking decreased from 46.7% to 39.5%, blood pressure treatment increased from 5.8% to 12.5% and systolic BP increased from 123.3 to 129.9mmHg.

hs-cTnI ranged from 0 to 173.0 ng/L at R1 to 0.3 to 164.6ng/L at R3. Median hs-cTnI levels changed from 2.6ng/L to 3.6ng/L to 3.4ng/L across the three rounds, with overall changes illustrated in Figure 2. hs-cTnI was above the LoD (>1.9ng/L) in 68.2% of participants at R1 (61.8% men, 38.2% women), 90.4% at R2 (50.8% men, 49.2% women), and 84.9% at R3 (55.1% men, 44.9% women). 95% of the changes in hs-cTnI from R1 to R3 are relatively small, between -3.5 and 6.48ng/L (Supplemental table 3). Higher levels of hs-cTnI were observed in men, but over time hs-cTnI increased in both genders. hs-cTnI values increased over time from R1 to R2 in the full cohort and continued to increase in CVD cases, reaching a plateau in non-cases from R2 to R3 (Supplemental figure 1).

Spearman's correlation coefficient for hs-cTnI from R1 to R3 ranged from 0.59 to 0.69. (R1 to R2 ρ=0.60, R2 to R3 ρ=0.69, R1 to R3 ρ =0.59). This range was similar although lower than correlations observed for SBP across three rounds (R1 to R2 ρ=0.76, R2 to R3 ρ=0.75, R1 to R3 ρ=0.67) or HDL cholesterol (range 0.75 to 0.81), correlations for BMI across rounds were higher (range 0.85 to 0.91). For the

prediction models, the number of participants for each risk set after exclusions is given in Supplemental table 4.

Association of history of change in hs-cTnI levels and CVD

During the follow-up period after round 3 (1993-94, median follow-up 16.6 years), 444 participants had a CVD event. hs-cTnI at R3 was a strong predictor of risk of CVD (HR 1.18 per interquartile difference in the cubic root of hs-cTnI (95% C.I.1.08, 1.30; p<0.001) after adjustment for cardiovascular risk factors (Model 2 in Table 2 and Supplemental table 5). The 10-year change in hs-cTnI (difference R3-R1), after adjustment for risk factors was also positively associated with CVD (HR 1.16; 95% C.I. 1.02, 1.31; p=0.023) (Model 3 in Table 2 and Supplemental table 5).

Association of longitudinal trend in hs-cTnI levels with CVD

During the follow-up after round 1 (median follow-up 27.5 years) 581 incident CVD cases were observed. After risk factor adjustment, hs-cTnI was positively associated with CVD in the joint model, with a hazard ratio of 1.31 (95% C.I. 1.15, 1.48), per interquartile difference in the cubic root; p<0.001 (Model 4 in Table 2 and Table 3). The Cox model with time dependent covariates yielded a HR of 1.22 (95% C.I. 1.12, 1.32) p<0.001 (Model 5 in Table 2 and Supplemental table 5). Both these models account for changing levels of cardiovascular risk factors and hs-cTnI at each round.

According to the longitudinal part of the joint model, hs-cTnI increased with age at round 1 by 0.007 (95% C.I. 0.006, 0.008, p<0.001) cube units and with time from baseline (coefficient 0.016, 95% C.I. 0.014, 0.017, p<0.001). hs-cTnI was

higher in men than women by 0.226 (C.I. 0.205, 0.247, p<0.001) cube units (Table 3).

Prediction modelling for troponin

The probabilities of 10-year risk of CVD were estimated from the Cox models based on 444 CVD events. The c-index for model 1 with cardiovascular risk factors was 0.744 (95% C.I. 0.717, 0.772). Adding a single most recent hs-cTnI measurement at round 3 (i.e. Model 2) to this, marginally improves prediction (c-index 0.750, improvement 0.0052 *p*=0.043) (Table 4). Model 3, replacing the last hs-cTnI measurement with the 10-year change in hs-cTnI, does not improve prediction upon the single measure of hs-cTnI (Model 2). Model 4, the JM incorporating the longitudinal trend in hs-cTnI and risk of CVD, improves prediction but only marginally when compared to Model 2. The c-index for Model 4 was 0.754 (95% C.I. 0.726, 0.782), improvement 0.004 *p*=0.03) compared to Model 2 (Table 4). The continuous NRI measure for Model 4 was 0.23 (p<0.001) when compared to Model 2.

Sensitivity analysis

Sensitivity analysis based on those with hs-cTnI and risk factors measured at round 3 (N=2339) are presented in Supplemental Tables 6-7. After risk factor adjustment, hs-cTnI was positively associated with CVD in the JM with a HR of 1.30 (95% C.I. 1.15, 1.47) per interquartile difference in cubic root of hs-cTnI (Supplemental table 6). The addition of hs-cTnI measured at round 3 to cardiovascular risk factors (Model 2) failed to improve prediction, based on 304 CVD events, (c-index improvement 0.004, p=0.089), when compared to Model 1, whose c-index was 0.755

(Supplemental table 7). None of the models incorporating change in hs-cTnI improved prediction when compared to the model with a single measure of hs-cTnI at round 3. (Supplemental Table 7). See Supplemental methods for further details.

Discussion

We questioned whether monitoring changes in troponin over time in general populations could lead to better prediction of future CVD events compared to a single measure of hs-cTnI. Our novel approach focused on 10-year risk of CVD, an established metric in CVD risk scores so we could make a realistic comparison between a single measure to longitudinal measures. We tested this in a longitudinal cohort with clear separation between measurement period (10 years of change in hs-cTnI) and follow-up period used for prediction (16 years) as prognostic models cannot use 'future' measures as predictors. Therefore a new 'baseline' at 10 years became the starting point for predictions. Our study had longer intervals of measurement than comparable studies (9, 10) and with larger number of events (9) and we used a joint modelling (JM) approach which more sensitively monitored the change in hs-cTnI (M4) than incorporating change as a predictor in the models (M3). We found that hs-cTnI increases over time in the general population and the change in hs-cTnI is associated with increased risk of fatal and non-fatal CVD. Our findings applied across a wide age range including a younger group, while previous findings for association of change in hs-cTnI or hs-cTnT with increased risk of heart failure, cardiovascular death and all-cause mortality are evident in older groups (>65 years) (9, 10) and a middle-aged group for coronary heart disease (13).We found three measures of hs-cTnI can be better than one in characterising risk of CVD and improving prediction. In terms of risk prediction in the general population, however,

the magnitude of the gain is minimal and offers no significant practical advantage over a single most recent measure of hs-cTnI for long term prediction of CVD risk.

The associations show that change in hs-cTnI is significantly linked with cardiovascular disease, confirming previous reports of association with cardiovascular mortality (10) and coronary heart disease (13). Rather than categorising troponin like these studies, which may reduce precision and power (29), we used a continuous measure. Our associations are significant and have tighter confidence intervals based on a sample of N=3178 compared to N=1797 (10) or N=3448 (13). Moreover the HR of continuous hs-cTnI is not directly comparable to the HRs of categorical variables. The associations showed that the change in hs-cTnI incorporated to the JM had a highly significant independent effect if added after other updated risk factors, highlighting its prognostic value independently of other risk factors. We quantified the impact of change in hsTnI compared to change in systolic BP on prediction of CVD (see supplemental methods). The change in SBP contributes twice as much to prediction of CVD than change in hsTnI (measured in relative contributions to the JM), which is unsurprising as SBP is a key modifiable risk factor for CVD, yet hsTnI eclipses some established risk factors such as HDL cholesterol and BMI in its impact. hs-cTnI and change in hs-cTnI offer the potential for identifying individuals at higher risk of CVD events but not currently identified by established risk factors.

Our statistical approach was sensitive enough to detect significant improvements in risk prediction by monitoring changes in hs-cTnI compared to a single measure. JM sensitivity may be a result of including the CVD outcome when

estimating the longitudinal hs-cTnI trend, which avoids diluting the association between hs-cTnI and CVD, while Cox time-dependent models tend to underestimate the true risk for hs-cTnI (30, 31). However, median hs-cTnI levels were relatively low from 2.6ng/L to 3.4ng/L over 10 years, but followed predictable trends. Levels frequently changed (7, 9, 10, 32), even though most had levels below diagnostic cutoffs for myocardial infarction (4, 7, 33). hs-cTnI levels across rounds were correlated and similar to other cardiovascular risk factors such as systolic BP but lower than BMI suggesting strong tracking over time. Detectable hs-cTnI levels increased from 68% at round 1 to 84.9% at round 3, consistent with levels in other general populations (2, 3, 4) and in a previous longitudinal study (7). hs-cTnI levels increased with age and it may be that the single last measure of hs-cTnI was sufficient for optimal prediction as this represented a time when hs-cTnI could become prognostically more relevant as the population aged. Other studies have found minimal benefit of incorporating change in hs-cTnI/hs-cTnT to prediction but have taken different statistical approaches and outcomes precluding a direct comparison. McEvoy et al. (13) compared a model with CVD risk factors incorporating the delta change in TnT from 2 measurements, 6 years apart, N=3448, including 622 CHD events. They found the addition of change in hsTnT offered no significant gain in discrimination (c-index difference 0.0004, *p* value 0.1). This is consistent with our adjusted model including delta change in hs-cTnI (Table 4, c-index difference -0.003, *p*=0.11) although the models are not directly comparable given the different troponin measurements and statistical approaches. However, they do find that incorporating the change in TnT improved prediction of heart failure and all-cause mortality (13). In older cohorts minimal predictive benefits have also been found. deFilippi et al. incorporated relative change in hsTnT over 2 years as a

covariate in a Cox model marginally improved classification (IDI) for heart failure and cardiovascular mortality but not c-index in an elderly cohort with ~8 years follow-up (10). Eggers et al. incorporated relative change in hs-cTnI over 5 years as a time-dependent covariate in a Cox regression with ~4 years follow-up, finding that this failed to improve discrimination of cardiovascular mortality (N=32 events) in an elderly cohort (>65 years) (9). Taking a similar approach in this cohort, the change in hs-cTnI was found to be less strongly associated with cardiovascular events (N=163) than the change in GDF15 or NTProBNP (11). The change in hs-cTnI was compared against baseline hs-cTnI or not formally compared prognostically against a single last measure (9, 10, 11). Our paper by comparison, has unequivocally tested whether the change in troponin adds prognostic information to 10-year risk models compared to a single hs-cTnI measure and has laid a framework for testing in other cohorts.

Missing data is a study limitation for longitudinal studies and it is difficult to distinguish data missing at random from missing not at random. Multiple imputation maximised the inclusion of relevant information for the prediction models. Omitting information from subjects with missing data can attenuate associations and cause bias (34), as we observed in a sensitivity analysis when we restricted the dataset to those available at round 3. We cannot exclude the possibility that sample degradation may have affected the biomarker measurements leading to overestimation of the change in hs-cTnI. However, previous studies have found that hs-cTnI assessment is robust to long term storage (3) and if specimens deteriorated at the same rate it should not affect the predictive value of hs-cTnI in the models

even if absolute levels are affected. Changes in medication use over 26 years may have affected the predictive value of hs-cTnI over time but little empirical data on such effects exist.

In summary, while a change in hs-cTnI improved prediction, it did not substantially improve estimates beyond a single most recent measure of hs-cTnI. A single measure of hs-cTnI is sufficient for 10-year prediction of CVD risk, simplifying the use of hs-cTnI as a stable prognostic marker for primary prevention of CVD and endorses prediction models of hs-cTnI for 10-year risk of CVD derived from other larger general population studies (2, 3). However, other factors such as physical activity (35), hyperglycemia (7, 36) and renal function (37) can influence changes in hs-cTnI. Further work will examine the potential for hs-cTnI to monitor changes in risk for the selection of higher risk subjects and targeting of therapy (6, 38).

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Annex: Sites and Key Personnel of the Contributing MORGAM Centres

Denmark

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Table captions

Table 1 Participant characteristics of the study population according to examination round they were collected in.

Table 2 Summary of the association between hs-cTnI and risk of CVD

Table 3 The joint model of longitudinal trend in hs-cTnI (Model 4)

Subcaptions:

Longitudinal submodel for hs-cTnI (from the joint model).

Survival submodel (from the joint model).

Table 4 Risk models describing the improvement of 10-year risk prediction for cardiovascular disease by high sensitivity troponin I in all participants.

582 Table 1 Participant characteristics of the study population according to examination

583 round they were collected in.

Risk factors	ROUND 1 (N=3785)	ROUND 2 (N=3672)	ROUND 3 (N=3461)
Examination age (years)	45.5±11.0	50.2±11.0	55.6±10.9
Gender (No. of men)	1940 (51.3%)	1867 (50.8%)	1726 (49.9%)
BMI (kg/m²)	24.6±3.9	25.2±4.0	26.0±4.3
Systolic BP (mmHg)	123.3±16.8	126.5±19.0	129.9±19.4
HDL cholesterol (mmol/L)	1.5±0.4	1.5±0.4	1.4±0.4
Total cholesterol (mmol/L)	5.8±1.2	6.1±1.2	6.2±1.1
Antihypertensive meds	221 (5.8%)	167 (4.6%)	434 (12.5%)
Diabetes	86 (2.3%)	121 (3.3%)	145 (4.2%)
Daily smoker	1768 (46.7%)	1829 (49.8%)	1366 (39.5%)
hs-cTnI (ng/L)	2.6 (1.6, 4.0)	3.6 (2.6, 5.0)	3.4 (2.3, 4.8)
Creatinine (mg/dL)	0.8 (0.7, 0.9)	0.8 (0.7, 0.9)	0.8 (0.7, 1.0)
CRP (mg/L)	1.2 (0.6, 2.8)	1.2 (0.6, 2.9)	1.5 (0.7, 3.5)

584

585 Mean (±SD), median (first quartile, third quartile) or Numbers (%). Data are derived

586 from the multiple imputed datasets. HDL high density lipoprotein cholesterol, BP blood

587 pressure, hs-cTnI high sensitivity troponin I, CRP c-reactive protein, eGFR estimated

588 glomerular filtration rate.

589

590

591 Table 2 Summary of the association between hs-cTnI and risk of CVD

592

Model	HR (95% C.I.)	p-value
Model 2 (hs-cTnI at R3)	1.18 (1.07, 1.30)	<0.001
Model 3 (hs-cTnI delta change R1, R3)	1.16 (1.02, 1.31)	0.023
Model 4 (joint model)	1.31 (1.15, 1.48)	<0.001
Model 5 (time dependent covariates)	1.22 (1.12, 1.32)	<0.001

593

594 Hazard ratios for hs-cTnI in Models 2 and 3 are based on 3178 individuals and 444

595 CVD events. Model 2 is based only on hs-cTnI at round 3. Hazard ratios for hs-cTnI

596 in Models 4 and 5 are based on 3702 individuals and 581 events. Models 4 and 5

account for changing levels of hs-cTnI and other risk factors. HRs are reported as per interquartile difference and adjusted for cardiovascular risk factors.

608 Table 3 The joint model of longitudinal trend in hs-cTnI (Model 4)

609 Longitudinal submodel for hs-cTnI (from the joint model).

	hs-cTnI$^{1/3}$ (95% C.I)	p-value
(Intercept)	1.004 (0.943, 1.065)	< 0.001
Time from baseline (years)	0.016 (0.014, 0.017)	< 0.001
Age at round 1 (years)	0.007 (0.006, 0.008)	< 0.001
Male	0.226 (0.205, 0.247)	< 0.001

610

611 Survival submodel (from the joint model).

	All (N=3702, 581 events) HR (95% C.I.)	p-value
hs-cTnI$^{1/3}$	1.31 (1.15, 1.48)	< 0.001
Male	1.73 (1.43, 2.09)	< 0.001
BMI (kg/m^2)	0.98 (0.96, 1.01)	0.15
Systolic BP (mmHg)	1.01 (1.01, 1.02)	< 0.001
HDL cholesterol (mmol/L)	0.62 (0.46, 0.82)	0.0012
Non-HDL cholesterol (mmol/L)	1.20 (1.12, 1.29)	< 0.001
Diabetes	1.73 (1.25, 2.40)	< 0.001
Daily smoker	1.80 (1.49, 2.17)	< 0.001

612

613 The joint model comprises two parts: The longitudinal submodel for the change in
614 hs-cTnI over time using a random intercept for hs-cTnI. hs-cTnI is incorporated into
615 the survival submodel via the estimate from this intercept. The survival (relative risk)
616 submodel incorporates the time dependent changes in hs-cTnI and other risk factors
617 across the 3 rounds. N number of participants and CVD events. HRs per interquartile
618 difference in the cubic root of hs-cTnI.

Table 4 Risk models describing the improvement of 10-year risk prediction for cardiovascular disease by hs-cTnI in all participants.

	all (N=3178)				
	c-index	difference	p value	cont. NRI	p value
Model 1 Cardiovascular risk factors (RF)	0.744				
Model 2 (hs-cTnI at R3) + RF	0.750	0.0052	0.043	0.194	0.043
Model 3 (hs-cTnI delta change R1, R3) + RF	0.746	-0.003	0.110	-0.162	0.033
Model 4 (hs-cTnI joint model) + RF	0.754	0.004	0.030	0.230	<0.001

Cardiovascular risk factors (RF) include sex, BMI, SBP, HDL and nonHDL-cholesterol, diabetes and daily smoking. R1 round 1, R3 round 3. Note that Models 3 and 4 are compared to Model 2 (i.e. Model 2 becomes the base model for these comparisons). Discrimination for 10-year risk prediction is based on 3178 individuals and 444 CVD events.

Figure captions:

Figure 1 Study design of the prospective Danish cohort.

Figure 1 Legend: Data were collected at three rounds of examination over 10 years with 26 year follow-up. Number of individuals attending exam are given, some individuals missed an exam and some had an incident event (indicated by abscent/blue). Number of exam participants, number of participants failing to attend exam plus numbers of missing data on covariates for those that did attend the exam. All individuals recruited at R1 were included in the multiple imputation model, see methods. Prognostic modelling; We compared a prognostic model with a single last measure of hs-cTnI and risk factors (smoking, blood pressure, prevalent diabetes, HDL and nonHDL cholesterol, BMI) to a model containing only risk factors. Then we compared the model with 10-year change in hs-cTnI and risk factors (incorporated into a joint model) to the model with single measure of hs-cTnI and risk factors.

Figure 2 Spaghetti plot visually illustrating the change in hs-cTnI over time.

Figure 2 legend: Each line represents a participant's hs-cTnI values changing over 3 rounds/10 years. The trend (blue line) shows an increase in hs-cTnI over time with increasing age in both genders. Lines shown are slightly transparent so that regions that appear darker have more points plotted on them. The blue line indicates a loess smoothing line of best fit to the data.