


Ultra-sensitive troponin I is an independent predictor of incident coronary heart disease in the general population

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Abstract Troponins are sensitive markers of myocardial injury and predictive of cardiovascular events, but conventional assays fail to detect slightly elevated troponins in a considerable proportion of the general population. Using a novel ultrasensitive assay, we explored the relationship of troponin levels with the incidence of coronary heart disease (CHD) in a case-cohort sample (mean age 52.5 ± 0.2 years, 51.5% women) comprising 803 CHD cases and 1942 non-cases. Ultrasensitive troponin I was detectable in 99.9% of available case-cohort samples. In an age- and sex-adjusted model, individuals in the highest quartile of the troponin distribution had a more than threefold increased risk for CHD events compared to those in the bottom quartile [hazard ratio,

HR, 3.11; 95% confidence interval (CI) 2.15–4.49]. In a model adjusting for cardiovascular risk factors including C-reactive protein, cystatin C and N-terminal pro brain natriuretic peptide, individuals in the highest troponin I quartile still showed a hazard ratio of 2.58 (95% CI 1.66–4.00) for incident CHD as compared to those in the lowest quartile. Ultrasensitive troponin I was detectable in almost all individuals of a study sample reflecting middle-aged to elderly European general population. Ultrasensitive troponin concentrations exhibit an independent, graded, positive relation with incident CHD.

Keywords Ultrasensitive troponin I · Coronary heart disease · Risk prediction · General population

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Introduction

Approximately 15 years ago, the introduction of troponin testing revolutionized the diagnosis of acute myocardial infarction (AMI) [1]. In the following years, more sensitive troponin assays were developed, allowing for an even earlier detection of myocardial infarction [2]. Several epidemiological studies demonstrated that serum troponin is not only useful for the diagnosis of AMI, but is also a measure of subclinical chronic myocardial injury and thus predictive of future cardiovascular events and mortality, including heart failure [3–6].

A few years ago, a novel ultra-sensitive troponin I assay has been introduced, further lowering the troponin detection threshold significantly [7]. This ultrasensitive troponin assay yields measurable troponin I levels in almost every individual, but epidemiological data are limited [6, 8]. We therefore investigated the relation of ultra-sensitive serum troponin I concentrations with incident coronary heart

disease (CHD) in the general population, based on a case-cohort design, derived from the MONICA/KORA Augsburg cohort study.

Materials and methods

Study sample

The Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)/Cooperative Health Research in the Region of Augsburg (KORA) studies served as the database for a prospective case-cohort study in initially healthy, middle-aged men and women [9, 10]. The MONICA Augsburg project was part of the multinational WHO MONICA project, and the design of both projects was described in detail elsewhere [11, 12]. Briefly, three independent population-based MONICA/KORA Augsburg surveys (S), with a total number of 13,427 participants (6725 men, 6702 women) were conducted in 1984/85 (S1), 1989/90 (S2) and 1994/95 (S3) in the city of Augsburg and the counties Augsburg and Aichach-Friedberg to estimate the prevalence and distribution of cardiovascular disease risk factors in men and women. A simple random sample within each 10-year age and sex group was selected in the city of Augsburg and a two-stage cluster sample was drawn in the two counties. Participants were aged 25–64 years in S1 and 25–74 years in S2 and S3. All participants were prospectively followed within the framework of KORA. The case-cohort design used in the present study has been described previously in detail [13].

Due to the low incidence of CHD under the age of 35, we restricted the source population to 10,718 persons (5382 men and 5336 women) between 35 and 74 years of age at baseline who participated in S1, S2 or S3. After exclusion of 1187 participants with missing blood samples, 231 participants with self-reported myocardial infarction, and one person who participated in two surveys, the source population for the present study comprised 9299 persons (4506 men, 4793 women).

For the case-cohort study, a random sample subcohort of 2163 participants (1154 men, 1009 women) was drawn from the source population, stratifying by sex and survey. This random sample was enriched with all incident CHD cases that occurred during the follow-up time until 2009, yielding a final case cohort sample of 2745 participants including 803 incident CHD cases, thereof 221 in the random sample subcohort. In contrast to the results of the case cohort studies described so far the follow-up period was extended for another 7 years from 2002 to 2009. Minimal follow-up information was available for all participants, however, 25.6% of the source population were lost to follow-up at some time during the follow-up period

and censored early. This includes all participants who moved out of the study area or reached the age of 75 years and did not actively respond to questionnaires anymore (12.6%) (because the MONICA/KORA myocardial infarction registry covers the study region and the age range up to 74 years only) and those who died of non CHD causes (13.0%). Median follow-up time was 14.7 years. All participants provided written informed consent. The study was approved by the ethics committee of the Bavarian Chamber of Physicians and complies with the principles outlined in the Declaration of Helsinki.

Assessment of cardiovascular risk factors

Cardiovascular risk factors were assessed as previously described in detail [14]. Total and high density cholesterol concentrations were measured using standard enzymatic assays. Alcohol consumption, smoking and physical activity were assessed using a standardized questionnaire.

Biomarker measurements

Ultrasensitive troponin I was measured in serum samples by technicians blinded to the clinical data using an ultrasensitivity, single-molecule counting assay (Erenna[®] Immunoassay System, Singulex, Alameda, CA) with a limit of detection of 0.04 ng/L⁸. The inter-assay coefficient of variation (CV) was 7%. NT-proBNP and cystatin C were measured by the same technology with inter-assay CVs of 8 and 25%, respectively. High sensitivity C-reactive protein (hsCRP) was measured using a high-sensitivity immunoradiometric assay (IRMA) (S1/S2: men aged 45–64/74 years; S3 all men and women) [15] or a high-sensitivity latex-enhanced immunonephelometric assay on a BN II (Siemens, Eschborn, Germany) (S1/S2: men aged 35–44 years, all women), Inter-assay CVs were 12.0 and 5.1%, respectively) [16]. Both methods gave similar results when the same samples were analyzed [17].

Endpoint definition and assessment

A combined endpoint that included incident non-fatal MI as well as fatal MI and sudden cardiac death (SCD) was used as the outcome variable (ICD 9: 410–414 und 798). Incident events were identified through follow-up questionnaires or through the MONICA/KORA registry of AMI [18, 19]. Until December 2000, the diagnosis of a major, non-fatal and fatal MI event was based on the MONICA algorithm [20], where a diagnosis of a major CHD event was based on symptoms, cardiac enzymes (creatin kinase, aspartate aminotransferase, and lactate dehydrogenase), and serial changes from 12-lead ECGs evaluated by Minnesota coding [21], necropsy results, and history of CHD in

fatal cases. Since January 1, 2001 all patients with MI diagnosed according to ESC (European Society of Cardiology) and ACC (American College of Cardiology) criteria were included [1]. Self-reported CHD events in participants, who were not part of the MONICA/KORA registry (because they were older than 74 years or had moved out of the original study area) were validated by information from hospital discharge letters or from the last treating physician. Deaths from myocardial infarction were validated by autopsy reports, death certificates, chart reviews, and information from the last treating physician.

Statistical analyses

Descriptive analyses for baseline characteristics were carried out for cases and non-cases. Weighting was performed using the survey and sex specific sampling weights. Missing values were imputed using 20-fold multiple imputation by chained equations (MICE) [22]. Means (geometric means for skewed distributions) or proportions for baseline characteristics were computed using the SAS procedure SURVEYMEANS. For categorical variables participants with and without incident CHD were compared using Wald Chi square test based on the SAS procedure SURVEYFREQ and imputation was accounted for using a method proposed by Schafer to combine multiple results of Chi square statistics [23, 24]. Corresponding tests for continuous variables were performed as t-tests on regression coefficients based on the SAS procedure SURVEYREG and were combined using Rubin's rules for multiple imputation [25, 26]. In case of non-normality, tests were carried out with log-transformed variables and results were presented as geometric means with antilogs of standard errors of the adjusted log-means. To investigate univariate associations between troponin and continuous risk factors for CHD, Pearson correlation coefficients were calculated in the random sample subcohort using Rubin's rules for multiple imputation.

To assess the relation of troponin with incident CHD, Cox proportional hazard regression was applied. For comparison of hazard ratios (HRs), sex-specific quartiles of ultrasensitive troponin I (based on the random sample subcohort) were coded with the bottom quartile as the reference category. The case-cohort design required correction of the variance estimation based on the sampling weights to give standard error estimates for the parameter estimates. Correction for standard errors was made using the method by Barlow [27]; incorporation of the additional variation due to imputation was performed using Rubin's rules for multiple imputation [25]. Tests for trend were conducted assigning the median value within each quartile to the corresponding quartile and including this variable in the Cox regression model. Multivariable adjusted Cox

models were calculated in five stages of adjustment: (1) adjustment only for age, sex and survey; (2) additional adjustment for cardiovascular risk factors derived from the Framingham risk score (i.e. smoking status, systolic blood pressure, total cholesterol, HDL-cholesterol and antihypertensive medication); body mass index, prevalent diabetes and for lifestyle factors, i.e. alcohol consumption (0, 0.1–39.9, ≥ 40 g/d for men; 0, 0.1–19.9, ≥ 20 g/d for women), physical activity (inactive, active) and BMI; (3) additional adjustment for circulating biomarkers of cardiovascular risk, i.e. NT-proBNP, cystatin C and hsCRP (all natural log-transformed for normality). Results are presented for each quartile of the troponin I distribution as HRs together with their 95% confidence intervals (95% CI). We additionally assessed the association of continuous natural-log transformed troponin I levels using the above mentioned model design.

Kaplan–Meier curves for incident CHD were calculated based on the random sample subcohort for comparison of the different quartiles of troponin I. Kaplan–Meier Curves are based on a randomly chosen single imputation.

The accuracy of the different models to assess 10-year event risk was evaluated by three measures: (1) the area under the receiver-operating characteristic curve (AUROC), also known as C-statistic; (2) the integrated discrimination improvement (IDI) statistics which can be viewed as the difference of the R^2 statistic between two models, i.e. the difference in the proportion of variance explained by the two models [28]; and (3) the category-free net reclassification index (NRI) [29]. Performance measures were calculated applying methods appropriate for survival data and case cohort design [30–32], which are implemented as SAS macros on <http://ncook.bwh.harvard.edu/sas-macros.html> [33].

For the correct assessment of performance, 1000 bootstrap samples were drawn from the original case cohort and were imputed using MICE. 95% CI were calculated using a bootstrap-based approach by Jiang et al. [34, 35]. For all analyses, a p value < 0.05 was considered to be statistically significant. Imputation was performed using R version 3.2.3 and R package mice version 2.25 [36, 37]. All statistical analyses were performed with SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA).

Results

Study sample

The characteristics of the study sample are provided in Table 1. Cases were older and had a higher prevalence of cardiovascular risk factors than non-cases. Ultrasensitive troponin I was detectable in 99.9% of available case-cohort

Table 1 Baseline characteristics of the study sample

Characteristic	Cases (n = 803)	Non-cases (n = 1942)	p value
Age (year)	59.2 (0.3)	51.9 (0.3)	<0.001
Women	30.9%	53.5%	<0.001
Survey			<0.001
S1	34.5%	28.0%	
S2	41.6%	26.2%	
S3	23.9%	25.8%	
Clinical risk factors			
Body mass index (kg/m ²)	28.4 (0.2)	27.0 (0.1)	<0.001
Prevalent diabetes	15.4%	4.2%	<0.001
Systolic blood pressure (mm Hg)	143.5 (0.7)	133.1 (0.5)	<0.001
Hypertension ^b	65.4%	40.4%	<0.001
Antihypertensive medication	33.1%	13.9%	<0.001
Lifestyle factors			
Alcohol intake			0.269
No (0 g/day)	30.3%	31.6%	
Moderate (<40/20 g/day)	46.5%	43.2%	
High (≥40/20 g/day)	23.2%	25.2%	
Physical activity			<0.001
Active	30.4%	38.5%	
Inactive	69.6%	61.5%	
Smoking status			<0.001
Never smoker	35.9%	48.7%	
Former smoker	33.3%	27.1%	
Current smoker	30.9%	24.2%	
Biomarkers			
Total cholesterol (mg/dl)	256.9 (1.67)	236.6 (1.02)	<0.001
HDL cholesterol (mg/dl)	51.30 (0.58)	57.34 (0.40)	<0.001
Ultrasensitive Troponin I (ng/L) ^a	2.56 (1.04)	1.49 (1.02)	<0.001
C-reactive protein (mg/l) ^a	2.37 (1.04)	1.41 (1.03)	<0.001
N-Terminal pro-BNP (pg/ml) ^a	290.2 (1.0)	226.5 (1.0)	<0.001
Cystatin C (mg/l) ^a	0.76 (1.02)	0.72 (1.01)	0.002

Categorical variables are presented as percentages and continuous traits as weighted arithmetic means (standard errors), unless indicated otherwise

^a Geometric mean (antilog of SE); ^b Hypertension (ISH-WHO 1999) or medically treated with known hypertension

HDL High density lipoprotein, *BNP* brain natriuretic peptide

samples. The 99th percentile of the randomly selected subcohort was 22.1 ng/L in men and 12.0 ng/L in women. The 75% percentile was 2.75 ng/L in men and 1.87 ng/L in women.

Ultrasensitive troponin I and markers of cardiovascular risk

The univariate correlations of ultrasensitive troponin I with selected markers of cardiovascular risk are provided in Table 2. Ultrasensitive troponin I levels were significantly (all $p < 0.01$) correlated with higher total cholesterol,

lower HDL cholesterol, higher systolic blood pressure, and higher concentrations of hsCRP, NT-proBNP, and cystatin (all $p \leq 0.002$).

Ultrasensitive troponin I and incident CHD

Figure 1 depicts unadjusted Kaplan–Meier curves for incident CHD, stratified by quartiles of the ultrasensitive troponin I distribution. The adjusted risk for incident CHD, stratified by quartiles of the ultrasensitive troponin I distribution, is given in Table 3. In a simple model adjusting only for age, sex and survey, we found a graded, positive

Table 2 Correlation between ultrasensitive troponin I^a and markers of cardiovascular risk in the randomly sampled subcohort

	r	p value
Total cholesterol	0.0798	0.0003
Age	0.4307	<0.0001
Body mass index	0.2499	<0.0001
Total cholesterol	0.0798	0.0003
HDL cholesterol	-0.1193	<0.0001
Systolic blood pressure	0.3704	<0.0001
C-reactive protein ^a	0.2258	<0.0001
N-terminal pro-BNP ^a	0.3801	<0.0001
Cystatin C ^a	0.1709	<0.0001

Data are Pearson correlation coefficients and the respective p-values

^a Natural log-transformed for normality

relation between even slightly elevated ultrasensitive troponin I and increased risk of incident CHD (p -trend < 0.0001). Individuals in the highest quartile of ultrasensitive troponin I distribution had a 3.11 fold higher risk for CHD than those in the lowest quartile. Multivariable models with progressive additional adjustment for potential confounders confirmed this observation. In a model adjusting for multiple cardiovascular risk factors including NT-proBNP, cystatin C and hsCRP (model 3), participants in the third quartile of the ultrasensitive troponin I distribution had a 1.56 fold increased risk for CHD,

and those in the highest quartile a 2.58 increased risk for CHD compared to individuals in the lowest quartile. Sex-specific analyses showed very similar results in men and women (data not shown). Sensitivity analyses on more restricted endpoints, i.e. (1) fatal MI and sudden cardiac death and (2) non-fatal MI were in line with the findings from our primary analyses (Supplementary Tables 1, 2).

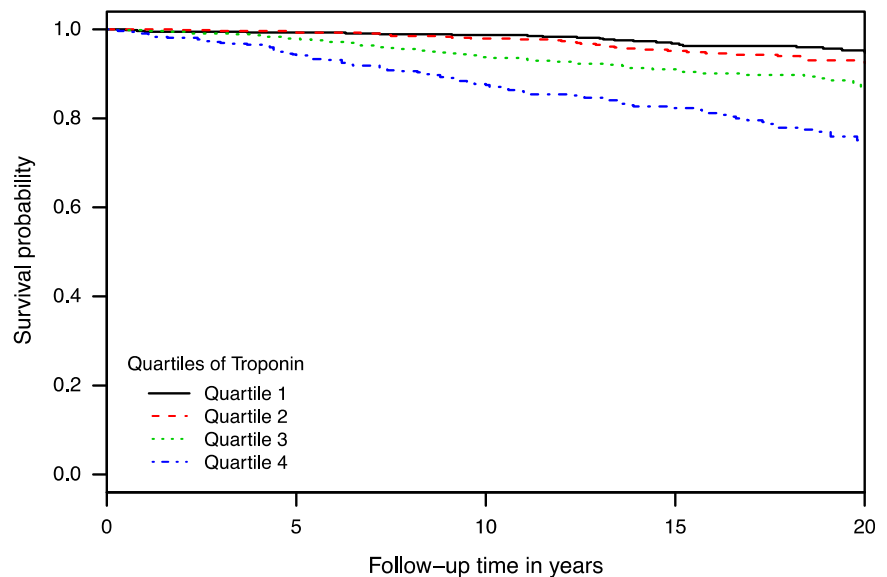
In order to assess the impact of very high troponin levels or severe renal impairment on our findings, we furthermore conducted a sensitivity analysis excluding individuals with ultrasensitive troponin levels above the 99th percentile and persons with an estimated GFR <30 mL/min. The results are presented in Supplementary Table 3, and did not substantially differ from our primary analysis.

Risk prediction performance assessment

The risk prediction improvement metrics for ultrasensitive troponin I are given in Table 4. Ultrasensitive troponin significantly increased the AUROC curve when added to a prediction model based on the Framingham risk score (delta ROC 0.006, p < 0.05). However, other metrics of risk prediction improvement, i.e. integrated discrimination improvement (IDI) and net reclassification improvement (NRI) were non-significant (p > 0.05).

When the sex-specific 75th percentile was used as a cut-off, ultrasensitive troponin had a positive predictive value (PPV) of 0.46 and a negative predictive value (NPV) of 0.78 for incident CHD. Similarly, using the sex-specific

Fig. 1 Kaplan Meier estimates for incident CHD, stratified by (sex-specific) quartiles of the ultrasensitive troponin I distribution, in the random subcohort



Number at risk:

Years	0	5	10	15
N	2163	2042	1852	1207
Events within 5 yrs	47 (2.2%)	64 (3.1%)	54 (2.9%)	37 (3.1%)

Table 3 Cox regression models: adjusted risk for incident CHD according to baseline levels of ultrasensitive troponin I

Quartile range (ng/L)	Quartiles of ultrasensitive troponin I						
	Q1	Q2	Q3	Q4			
Women	0.05–0.80	0.80–1.20	1.20–1.87	1.87–495.68			
Men	0.05–1.22	1.22–1.77	1.77–2.75	2.75–107.44			
Hazard ratios		<i>p</i> for trend		Continuous ultrasensitive troponin ^d	<i>P</i>		
Model 1 ^a	1.0	1.32 (0.89–1.95)	1.83 (1.25–2.69)	3.11 (2.15–4.49)	<0.0001	1.73 (1.48–2.03)	<0.0001
Model 2 ^b	1.0	1.39 (0.92–2.11)	1.60 (1.06–2.43)	2.72 (1.79–4.12)	<0.0001	1.48 (1.20–1.83)	0.0002
Model 3 ^c	1.0	1.37 (0.91–2.07)	1.56 (1.03–2.38)	2.58 (1.66–4.00)	<0.0001	1.45 (1.15–1.83)	0.0016

Hazard ratios and their 95% confidence intervals were derived from Cox regression models

^a Model 1 adjusted for age, sex and survey

^b Model 2 adjusted for Framingham risk factors (i.e. smoking status, systolic blood pressure, total cholesterol, HDL-cholesterol and antihypertensive medication), body mass index, prevalent diabetes, alcohol consumption and physical activity

^c Model 3 adjusted for factors in model 2 + NT-pro-BNP, Cystatin C and hsCRP

^d Natural log-transformed

Table 4 Risk prediction performance measures

	Test performance (95% CI)
ROC basic model ^a	0.825 (0.809, 0.841)
ROC extended model ^b	0.831 (0.815, 0.847)
ROC improvement	0.006 (0.001, 0.010)
NRI (continuous)	0.211 (–0.022, 0.396)
IDI	0.034 (–0.064, 0.081)

ROC Area under the receiver operating curve, NRI net reclassification improvement, IDI integrated discrimination improvement

^a Basic Model adjusted for sex, age, survey and Framingham risk factors, i.e. smoking status, systolic blood pressure, total cholesterol, HDL-cholesterol and antihypertensive medication

^b Extended Model additionally adjusted for ultrasensitive troponin

99th percentile as cut-off, ultrasensitive troponin I had a PPV 0.54 and an NPV of 0.71 for incident CHD.

Discussion

Using a novel ultrasensitive troponin I assay, we investigated the relation of circulating troponin I concentrations with incident CHD events in a large sample derived from the general population. Ultrasensitive troponin was measurable in almost all individuals. We observed that even at concentrations far below the 99th percentile (the standard cut-off used in clinical practice), there is a graded positive association between ultrasensitive troponin I and increased risk of incident CHD. This association was consistent and persisted after adjustment for multiple cardiovascular risk factors.

Existing literature

Several studies have assessed the relation of circulating troponin I levels with future cardiovascular risk. However, even high-sensitive troponin assays fail to detect troponin I in a considerable proportion of the general population. For high-sensitive troponin T, detectable levels have been reported in 25–68% of the general population [3, 38, 39], for high-sensitive troponin I approximately 80–95% have detectable levels [39, 40]. In the JUPITER trial cohort, troponin I was detectable in 92% of participants, using a high sensitivity troponin I assay with a lower detection threshold of 1.9 ng/L, and was an independent predictor of cardiovascular events [41]. Based on the same high-sensitive troponin I assay, a Europe-wide analysis of several studies confirmed that troponin I was independently associated with cardiovascular disease, and also improved measures of risk prediction [42].

The ultrasensitive assay used in the present analysis has an almost 50-fold lower threshold of detection, and there is only limited data on ultrasensitive troponin I levels in the general population. Using a nested case–control sample (211 cases, 253 controls) derived from the Minnesota Heart Study, Apple et al. reported an association of ultrasensitive troponin I with cardiovascular death [43], with detectable troponin levels in 100% of the study sample. Neumann et al. found that, using the same ultrasensitive troponin I assay, troponin levels were detectable in 94% of a large sample reflecting the Finnish general population [8]. The authors observed a significant association of troponin I levels with myocardial infarction, stroke, incident heart failure, and incident cardiovascular disease. However, their analyses were not adjusted for other serum biomarkers of cardiovascular risk such as hsCRP or natriuretic peptides. Ultrasensitive troponin measurements did

not improve risk prediction (assessed by NRI) in the overall cohort [8]. Wang et al. assessed ultrasensitive troponin I levels in 2428 participants of the Framingham Heart study [6]. They found detectable troponin in 81% of the study sample. Troponin concentrations were associated with death, cardiovascular events and incident heart failure, but not with coronary events (possibly due to insufficient power). The contribution of ultrasensitive troponin I to risk prediction was not assessed dedicatedly in that study (only as part of a multi-marker score).

To our knowledge, the present study is the first to demonstrate that even slightly elevated troponin I levels measured by an ultrasensitive assay are associated with incident CHD in the general population, largely independent of traditional risk factors, lifestyle factors, and other established serum biomarkers of cardiovascular risk (i.e. hsCRP, NT-proBNP and cystatin C).

Clinical relevance

Our data suggest that ultrasensitive troponin I measurements do not relevantly improve cardiovascular risk prediction metrics as assessed by AUROC, IDI and NRI. This lack of risk prediction improvement may be attributed to the excellent performance of the Framingham risk score variables in our study sample (AUROC 0.8). Nevertheless, individuals in the highest ultrasensitive troponin I quartile (>1.87 ng/L in men and >2.75 ng/L in women) had an approximately threefold—or 200% increased—risk for incident CHD as compared to those in the lowest quartile. This represents a clinically highly relevant observation: a similar increase in cardiovascular risk is associated with prevalent diabetes or cigarette smoking [44, 45]. Thus, high ultrasensitive troponin I levels can be considered a strong risk factor and should trigger strict measures of cardiovascular prevention.

Limitations

Some limitations of our study should be mentioned. First, our study is observational in nature, and causal inferences thus cannot be drawn. Second, ultrasensitive troponin I levels were measured only once. This may introduce a random error driving our results towards the null hypothesis (of no association between ultrasensitive troponin and CHD). Third, our sample consists of middle-aged to elderly Caucasians. Thus, the applicability of our findings to other ethnic and age groups or to certain patient groups remains to be elucidated.

Conclusion

Ultrasensitive troponin I was detectable in almost all individuals of our random sample reflecting the general population in Western Europe. Ultrasensitive troponin

concentrations exhibit a graded, positive relation with incident CHD, largely independent of established cardiovascular risk factors, including markers of systemic inflammation, renal function, and natriuretic peptides. Enforced preventive measures may be applied to individuals with elevated ultrasensitive troponin I levels.

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Compliance with ethical standards

Conflict of interest WK reports consultant or advisory roles for Novartis, Pfizer, The Medicines Company, Amgen, AstraZeneca, MSD, GSK, KOWA and DalCor; honoraria from Astra Zeneca, Novartis, MSD, Amgen, Actavis, Sanofi and Berlin-Chemie; research contracts with Abbott, Roche diagnostics, Beckmann and Singulex. JT is employee, chief scientific officer and stock options holder of Singulex, the company that produces the ultrasensitive troponin I assay. SW is employee of Roche diagnostics. The other authors report no conflicts of interest. The funding organizations played no role in the design of study, choice of enrolled individuals, review and interpretation of data, and final approval of manuscript.

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