Risk for cardiovascular events responds nonlinearly to carotid intima-media thickness in the KORA F4 study

Cristoforo Simonetto[1], Margit Heier[2], Susanne Rospleszcz[2], Christa Meisinger[2,3], Cornelia Then[4,5], Jochen Seißler[4,5], Annette Peters[2,6], Jan Christian Kaiser[1,*]

[1]Helmholtz Zentrum München, Institute of Radiation Medicine, 85764 Oberschleißheim, Germany

[2]Helmholtz Zentrum München, Institute of Epidemiology, 85764 Oberschleißheim, Germany

[3]Chair of Epidemiology, Ludwig-Maximilians-University München, UNIKA-T Augsburg, Augsburg, Germany

[4]Diabetes Centre, Medizinische Klinik und Poliklinik IV, Klinikum der Ludwig-Maximilians-Universität, Munich, Germany

[5]Clinical Cooperation Group Diabetes, Ludwig-Maximilians-Universität München and Helmholtz Zentrum München, Munich, Germany

[6]Chair of Epidemiology, Ludwig-Maximilians-University München, Munich, Germany

*To whom correspondence should be addressed. Email: christian.kaiser@helmholtz-muenchen.de

Abstract

Background and aims

Risk assessment studies on the impact of carotid intima-media thickness (CIMT) on cardiovascular events (CVEs) often apply a linear relationship in Cox models of proportional hazards. However, CVEs are mostly induced through rupture of plaques driven by nonlinear mechanical properties of the arterial wall. Hence, the risk response might be nonlinear as well and should be detectable in CVE incidence data when associated with CIMT as surrogate variable for atherosclerotic wall degeneration.

Methods

To test this hypothesis, we investigate the KORA F4 study comprising 2580 participants with CIMT measurements and 153 first CVEs (86 strokes and 67 myocardial infarctions). CIMT is only a moderate predictor of CVE risk due to confounding by attained age. Biological evidence suggests that age-related CIMT growth is not entirely connected with atherosclerosis. To explore the complex relations between age, CIMT and CVE risk, we apply linear and nonlinear models of both CIMT and dnCIMT, defined as deviation from a sex and age-adjusted normal value.

Results

Based on goodness-of-fit and biological plausibility, threshold and logistic step models clearly reveal nonlinear risk response relations for vascular covariables CIMT and dnCIMT. The effect is more pronounced for models involving dnCIMT as novel risk factor, which is not correlated with age.

Conclusions

Compared to the standard approach of risk assessment with linear models involving CIMT, the application of excess dnCIMT with nonlinear risk responses leads to a more precise identification of asymptomatic high risk patients, especially at younger age.

Key words: subclinical atherosclerosis, carotid intima-media thickness, stroke, myocardial infarction, risk stratification

Introduction

Atherosclerosis causes the majority of strokes and myocardial infarctions (MIs), which are jointly considered here as cardiovascular events (CVEs). Development of atherosclerosis begins early in life with deregulated lipid metabolism without causing clinical symptoms for decades[1]. Progression to chronic inflammation in the vascular system might prompt changes in mechanical properties of arteries[2]. Blood flow in the artery is strongly influenced by nonlinear wall elasticity[3]. Vessel elasticity is impaired by mechanical risk factors such as thickness and calcification. CVEs are typically induced through rupture of plaques in the arterial wall[4]. Sometimes rupture of intracranial aneurysms may cause strokes in particular subarachnoid haemorrhages. Wall rupture is a critical phenomenon characterised by a sudden transition brought about by continuously deteriorating stability. These biomechanical observations motivate the search for a nonlinear response of CVE risk to atherosclerotic wall degeneration represented by carotid intima-media thickness (CIMT) as surrogate variable.

CIMT is a well-studied biomarker of subclinical atherosclerosis which is best measured with ultrasonography[5]. It increases steadily with attained age, and to a lesser extent is associated with other traditional risk factors such as body mass index (BMI), hypertension (HT) and dyslipidaemia (DL)[6]. Positive statistical associations between CVE risk in the general population and CIMT based on linear models have been reported in numerous studies (reviewed in Stein et al.[7]). CIMT is considered a moderate linear predictor of CVE risk in Cox models of proportional hazards[8]. Plaques appear later in life and increase the CVE risk complementary to excessive CIMT[5, 9].

Already at young age, increased CIMT is associated with various cardiovascular risk factors[10]. Even adults younger than 45 years exhibit an elevated CVE risk related to an overall moderate CIMT[11]. Hence, could *excessive* CIMT compared to an age-adequate value predict the risk more reliably? Moderate increase in CIMT may not be necessarily induced by atherosclerosis[12, 13]. A low pace of growth in CIMT could simply reflect equilibrium of pressure and flow in the arteries. Only beyond a

certain level, CIMT would represent atherosclerotic degeneration[14]. Altogether, these findings point to a complex and possibly nonlinear relation between CIMT, attained age and CVE risk.

Many studies have been concerned with classification of CIMT measurements into normal and pathological values based on either fixed cut points or percentiles (reviewed in Bauer et al.[5]). However, nonlinearity of the CIMT-risk relation was rarely in the focus of pertinent investigations[8]. Applying different functional forms of risk response, our statistical association analysis is guided by the hypothesis that nonlinear effects of excessive CIMT leave imprints in the age-related risk pattern for CVEs. To this aim, we analyse the population-based Cooperative Health Research (KORA) F4 study from the Region of Augsburg in Southern Germany.

Materials and methods

Study population

The Cooperative Health Research in the Region of Augsburg (KORA) F4 study comprises data collected in 2006–2008. The KORA F4 study is the first follow-up of the KORA S4 study (1999-2001), which is a population-based survey conducted in the region of Augsburg in southern Germany. KORA F4 comprises 3080 of the original 4261 KORA S4 participants. Age at baseline was 25 to 74 years. Further details of the study design are given in Rathmann et al.[15] and Meisinger et al.[16]. From KORA F4 we excluded subjects with retroactively withdrawn consent, with CVEs prior to S4 examination, with missing covariables and without valid CIMT measurements, thus attaining a final sample size of N = 2580 (Supplementary Fig. 1).

Ethics statement

Investigations were carried out in accordance with the Declaration of Helsinki, including written informed consent of all participants. All study methods were approved by the ethics committee of the Bavarian Chamber of Physicians, Munich.

Definition of outcome "Cardiovascular Event" (CVE)

The outcome "Cardiovascular Event" (CVE) comprises the following diseases: incident fatal or non fatal MI, sudden cardiac death and fatal or non fatal stroke.

Fatal, non-fatal MIs and coronary deaths (cardiac cases) of F4 participants were identified by the MONICA/KORA coronary event registry if they fulfilled the inclusion criteria[17]. Until December 2000, the diagnosis of a major non-fatal MI was based on the MONICA algorithm taking into account symptoms, cardiac enzymes, and ECG changes[18]. Since January 2001, all patients with MI diagnosis according to ESC and ACC criteria were included[19, 20]. Coronary deaths were validated by death certificates, autopsy reports, chart reviews, and information from the coroner or the last treating physician.

Non fatal-strokes including transient ischemic attacks (TIA) and non-fatal MI, which were not registered in the MONICA/KORA coronary event registry for reasons of living outside the study region or age older than 74 (84) years, were assessed by questionnaires in postal follow-up. Using data from participants hospital records and information gathered from their attending physicians, all self-reported potential incident stroke/MI cases and the date of diagnosis were validated. The bias from undetected non-fatal CVEs (false negatives) cannot be quantified but other studies showed a negligible effect [21].

To ascertain fatal strokes and coronary deaths the survival status of all participants was regularly checked based on information provided by population registries inside and outside the study area. Death certificates of deceased participants, preserved by local health authorities, were analyzed and the main causes of death were extracted. The following three-digit International Classification of Diseases, Ninth Revision (ICD-9) codes were considered in German modification as death due to Cardiovascular disease: 410-414, 430, 431, 433, 434, 436 and 798.

In total 153 CVEs, broken down into 67 MIs and 86 strokes (subdivided into embolic stroke N=17, ischaemic stroke N=34, intracerebral haemorrhage N=7, subarachnoidal haemorrhage N=5, TIA/PRIND N=17, unkown stroke type N=6) were included in the analysis.

Measurement of CIMT

Ultrasound measurements of the extracranial carotid arteriae have been previously described[22, 23]. Briefly, all measurements were conducted by two sonographers according to a standardised protocol as used in the Rotterdam study[24]. Compliance with the protocol is generally considered as sufficient to ensure measurement reproducibility. Optimal images of both common carotid arteries (CCA) were identified and stored on DVD. Consequently, CIMT was ascertained off-line over a length of 10 mm beginning at 0–5 mm of the dilatation of the distal CCA. To this aim, an automated edge detection reading system (Prowin software, Medical Technologies International, USA) was used. The final CIMT value was calculated as the average of the measurements of 3 frozen images from both the left and

right CCA. As previously reported, measurements of inter-sonographer (n = 30 CIMT measurements) and inter-reader variations (n = 50 CIMT measurements) showed coefficients of variations of 1.9% and 3.0% and Spearman correlation coefficients of ≥ 0.89[23]. The intra-sonographer repeatability (mean differences (SD) in IMT thickness of CCA) between two ultrasonic scans was 0.013 mm (0.018) (n= 50 patients with 300 scans).

Measurements of anthropometric and clinical risk factors

Information on smoking behaviour and alcohol consumption (AC) was collected via a standardised interview conducted by trained medical personnel.

Smokers were classified as heavy smokers if they had accumulated more than ten packyears and as light smokers if they had accumulated ten packyears or less. A regular smoker was defined as a participant who smoked at least one cigarette per day. Interview information on AC was translated into individual intake estimates given in g/day.

Systolic and diastolic blood pressures were measured by use of an oscillometric digital blood pressure monitor (HEM-705CP, Omron Corporation, Tokyo, Japan) three times at the right arm of seated participants, after at least five minutes at rest. The pause between readings was three minutes. A cuff size of 14x48 cm was used for upper arm circumferences up to 32 cm and a cuff size of 16x65 cm for upper arm circumferences exceeding 32 cm. The mean of the second and third measurement was calculated and used for the present analyses. Hypertension (HT) was defined as blood pressure values ≥ 140/90 mm Hg and/or use of antihypertensive medication, given that the individuals were aware of being hypertensive.

Anthropometric measurements were taken after participants had removed shoes, heavy clothing, belts and corsets. Body weight was measured in light clothing to the nearest 0.1 kg and height to the nearest 0.5 cm. Body mass index (BMI) was calculated as weight in kg divided by height in meters squared.

Dyslipidemia (DL) was defined as a ratio of total serum cholesterol to HDL cholesterol of 5 or higher. Total cholesterol and HDL cholesterol were assessed by CHOD-PAP methods (CHOL Flex, and AHDL Flex).

The status of statin intake (SI) (yes/no) for S4 and F4 participants was determined in a pre-planned interview. Particpants were asked to bring all medication along with them, which they have taken during the past 7 days preceding the interview. Medical staff registered the medication data online using the IDOM software[25]. The drug class "statins" was categorized according to the Anatomical Therapeutical Chemical (ATC) classification index.

CIMT decomposition

To evaluate non-parametric age trends the data set was split into 20 subsets for the two sexes and ten age groups < 35, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74, > 74 yr. For each subset arithmetic means of CIMT and AaE were calculated (Table S1). Inspection of the 20 paired means, plotted against AaE in Supplementary Fig. 3, suggested a linear growth of CIMT for both sexes. To define a normal CIMT for a "healthy" person without any of our available risk factors elevated at a given age, sex-specific linear regression on CIMT was performed (Supplementary Fig. 2). By subtracting normCIMT(sex, AaE) from the individually measured CIMT values the new auxiliary covariable dnCIMT is obtained for each study participant. Fig. 1 depicts boxplots of dnCIMT per age group. Note, that dnCIMT is defined as the *synchronous* deviation from normCIMT. Examples of dnCIMT calculation for a woman at AaE 50 yr and man at AaE 65 yr are visualised in Supplementary Fig. 3. Pairwise Pearson correlation coefficients for covariables AaE, CIMT, dnCIMT and normCIMT are given in Supplementary Fig. 4

Follow-up of participants

Begin of follow-up was age at entry in the F4 study except for 151 participants for whom begin of follow-up was set to age at entry into S4 (Supplementary Fig. 1). These 151 participants remained members of the F4 study although their follow-up ended either between S4 and F4 due to CVE (N =

58) or at begin of the F4 study (N = 93). In this case for all covariables except CIMT the corresponding S4 measurements were used. CIMT values at S4 have been estimated by back extrapolation of F4 measurements based on the non-parametric growth curves depicted in Supplementary Fig. 3. Back extrapolation led to a small reduction of CIMT values between 2% and 12% with negligible influence on the CIMT distribution. We found it important to keep these 151 members of the F4 study since they contribute valuable information of CVE risk at younger age. Their mean age at CVE was 63 yr compared to 70 yr for the reduced analysis sample (N = 2429). To control for a possible bias we repeated the main analysis of the present study with the reduced sample. All results were confirmed although effects on HRs appeared attenuated with larger confidence intervals (CIs) and p-values.

All 2580 participants were followed up until their first CVE (endpoint), death from other causes than CVE (censored), dropout from the study (censored) or entry in the subsequent FF4 study (censored). Maximal follow up was 8.5 years. Median follow-up for participants without CVE was 6.5 years, whereas median follow-up for participants with CVE was 3.9 years.

Summary of analysis sample

Demographics, clinical risk factors and CIMT-related variables of the analysis sample are characterised in Table 1. Differences in covariables between F4 participants included in the analysis sample compared to excluded F4 participants are summarised in Table S2.

Statistical analyses

Cox proportional hazard models were applied to identify possible nonlinearities in the relationship between CIMT or dnCIMT and CVE risk[26]. In a first step univariate regression with covariables X = CIMT or dnCIMT was applied to explore linear and nonlinear relationships. Motivated by the trend of crude HRs in categories of dnCIMT (Fig. 2) or CIMT (Supplementary Fig. 5), three models for the dependence of the hazard ratio (HR) on X were tested

1. linear: ln(HR) ~ X,

2. threshold: ln(HR) = 0 for $X < X_{thr}$, ln(HR) ~ X for $X \geq X_{thr}$

3. logistic step: ln(HR) ~ 1/2 (1 + tanh[$(X-X_{cen})/X_{slp}$]).

For the threshold model the threshold parameter X_{thr} is subject to optimisation. For the logistic step model the slope X_{slp} is fixed to 0.02 mm to ensure a smooth step whereas the centre of the step X_{cen} is subject to optimisation. Optimal values for the vascular parameters X_{slp} and X_{cen} are obtained by stepwise search of the minimum for the likelihood function with step size 0.01 mm (Supplementary Fig. 6). Hence, threshold and logistic step models contain one adjustable parameter more compared to linear models which is reflected in the evaluation of goodness-of-fit. Other models with loglinear, exponential or U-shaped risk relations have been investigated in scoping calculations for the present analysis. Results are not reported here to keep the main analysis concise.

After identification of plausible risk models in univariate analysis the models were tested with sex and AaE-adjustment and as multivariable models with adjustment for all available covariables listed in Table 1. AaE was centered at 55 yr in units of 5 yr increase, BMI was centred at 25 kg/m^2.

Goodness-of-fit was measured by the Akaike Information Criterion AIC = deviance + 2*no. of covariables of the corresponding Cox model. Concordance Area Under Curve (AUC(t)) is given as Supplementary information. Detailed results of the multivariate analysis are shown in Supplementary Tables 3-8.

All statistical analyses including Cox regression were performed with the R software package[27]. *p*-values < 0.05 denote statistical significance.

Results

CIMT decomposition

Boxplots of Fig. 1 display the frequency distributions of dnCIMT for the twenty sex and AaE-adjusted subsets.

A correlation of r = 0.67 reveals AaE as a confounding variable for CIMT (Supplementary Fig. 4). On the other hand, dnCIMT is *very weakly* correlated to AaE (r= 0.06), so that confounding by AaE is avoided in models involving dnCIMT. The rather strong correlation of CIMT with dnCIMT (r = 0.77) ensures a representation of similar properties independent of AaE in CIMT and dnCIMT.

The assumption of linearity for normCIMT increase was tested with cut points at AaE of 45, 50 and 55 yr. Age cut points did not improve the total deviance of linear regression on CIMT. Simple Z-tests of growth rates before and after cut points yielded marginal significance for women and no significance for men. Menopause might have a weak influence in CIMT growth, which has been neglected here. Hence, the decomposition of CIMT = dnCIMT + normCIMT produces an important disentanglement, which we exploit for CVE risk assessment.

Univariate analysis

Using dnCIMT as single covariable revealed a clear hierarchy of models when judged by the AIC. Fig. 2 shows the corresponding HRs as functions of dnCIMT. To visualize the uncertainty coming with the limited number of cases in the analysis sample crude HRs have been plotted as points with CIs into Fig. 2. The points can be connected to a U-shaped profile with increasing risk on the two ends of value space for dnCIMT. Since the negative slope in HR for negative dnCIMT was not significant ($p > 0.3$) in multivariate analysis, we did not further examine the potential risk posed by the thinning of arterial walls.

Univariate Cox regression involving CIMT identified the linear model as markedly superior to the logistic step model (Supplementary Fig. 5). A threshold in CIMT could not be found.

Multivariate analysis

HR estimates for all baseline covariables were comparable between models involving CIMT and those involving dnCIMT (Supplementary Tables 3-8). The strongest predictor of CVE risk is AaE. The corresponding HR increases by a factor of about 1.3 in 5 yr. The HR for women reached only 60% of the HR for men. AC and BMI confer no significant CVE risk with HR estimates close to 1. Positive markers DL+ or ST+ increase or decrease the HR by factors 4/3 or 3/4 but without statistical significance. The HR for light smokers was not enhanced but heavy smokers exhibit a HR twice as high compared to non-smokers. A positive hypertension marker HT+ increases the risk significantly by a factor of 2.4. Table 2 summarises the main results for the linear models and the preferred logistic step models involving CIMT and dnCIMT as vascular covariables.

For models involving dnCIMT, multivariate analysis confirms the observations from univariate analysis on the nonlinear risk dependence although the associations appear attenuated with higher p-values. Increasing the number of covariables also reduces the depth of minima for the likelihood functions as demonstrated in Supplementary Fig. 5 for the threshold parameter (optimal value dnCIMT = 0.02 mm) of the threshold model and the centre of the step (optimal value dnCIMT = 0.09 mm) in the logistic model.

Models involving CIMT did not produce such a clear pattern of results. The logistic step model involving CIMT improved goodness-of-fit only if AaE is applied as additional covariable but not in univariate regression. The optimal step of CIMT in multivariate regression was centered at 1.01 mm in the logistic model. The corresponding search for a threshold in CIMT was not successful.

Fig. 3 depicts a risk profile of the analysis sample in a CIMT – age framework. The scatter plot of CIMT values measured at AaE separates study participants of low CVE risk (HR ≤ 2) and high risk (HR > 2) according to the logistic step model involving dnCIMT.

In Supplementary Fig. 7 risk profiles of the linear model involving CIMT and the logistic step model involving dnCIMT are compared to visualise model-specific age-risk patterns of F4 participants with elevated risk (HR > 1.2).

Discussion

Linear models involving CIMT or dnCIMT with adjustment for all available covariables produced HRs × standard deviation (Table 1) of 1.24 (95% CI 1.03-1.51) or 1.25 (95% CI 1.09-1.43). Our estimates are in line with HR estimates for CVEs from previous studies summarised by Stein et al.[7] (their Table 1).

Models involving dnCIMT provide a slightly better description of the data in terms of AIC compared to models involving CIMT. Footprints of dnCIMT-related nonlinearity of the CVE remained visible in the incidence data after full adjustment. For the threshold model in multivariate analysis the linear predictor in the region of positive dnCIMT is about twice as high compared to the predictor for the whole range of dnCIMT values (Supplementary Tables 6 and 7). These findings point to higher vessel vulnerability if CIMT exceeds its adjusted normal value (normCIMT in Supplementary Fig. 3). Whereas linear CIMT models produce an ever increasing risk with increasing CIMT, our nonlinear models involving dnCIMT suggest that a notable CVE risk occurs only for CIMT values above a normal CIMT pertaining to persons without elevated risk factors, as proposed already by Bots et al.[14]. Our model results exhibit a plausible upper limit for "risk-free" CIMT values slightly above the adjusted normCIMT (i.e. dnCIMT between 0.02 – 0.09 mm). Confidence intervals of estimates for the step centre and the threshold can be approximated by inspection of Supplementary Fig. 6.

Chambless et al.[28] report nonlinear response relations between CIMT and incident clinical stroke. They applied cubic splines in addition to a linear response and found a plateau of the HR at CIMT > 1 mm for men and an attenuation of the slope for women. Although their estimated HRs were sex-specific and much larger as those from the present study they arrive at the same conclusion that linear responses underestimate the risk association at younger age.

The processes leading to atherosclerosis are understood quite well, but a comprehensive mathematical model linking the development of arterial lesions and plaques further to wall rupture and CVE risk is still lacking. As a starting point a mechanistic model based on gradual pathologic

transformation of arteries has been proposed recently[29]. Insights on CIMT-risk relations from the present study can help to improve the conceptual design of such mechanistic models.

Limitations of our study pertain to the low number of cases which caused large uncertainties in crude HR estimates (Fig. 2) and prevented to determine the functional form of the dose response more precisely. Furthermore, we do not have specific information about medication compliance or statin type (standard or strong).

Relevance of nonlinearity

On a more general note, the application of linear vs. nonlinear models for risk assessment makes a notable difference when the cardiovascular system is influenced by planned intervention i.e. for patients undergoing radiation therapy (RT). For example, RT is known to increase CIMT in head and neck cancer patients[30]. After exposure to high therapeutic radiation doses of about 50 Gy, CIMT was measured several years later markedly enhanced by about 0.1 mm on average[31]. Other atherosclerotic risk factors had no impact on CIMT after RT. Based on the results of Table 2 the RT-related CIMT growth would increase the CVE risk by a HR of 1.2 independent of the actual CIMT status according to the linear CIMT model. On the other hand, the nonlinear responses of the present study suggest no risk increase if CIMT values remain below a normal CIMT. Shifting CIMT above the sex and age-specific normal value would lead to a risk markedly above the linear prediction. The risk profile displayed in Fig. 3 illustrates the relevance on nonlinearity if we assume that F4 participants (identified in group 2) with otherwise uncritical dnCIMT had entered the high risk zone by an RT-related increase of CIMT by 0.1 mm.

For risk projection with a linear response knowledge on the state of disease progression is not necessary. The risk from increasing CIMT is independent of the atherosclerotic state. However, if the nonlinear responses proposed in the present study bear some significance, such knowledge is required. Now the risk from an increasing vascular covariable depends on its initial value. Hence, based on goodness-of-fit nonlinear models taking into account the individual state of the disease

provide a moderately improved risk projection compared to linear models, which do not rely on such information.

Benefit of CIMT decomposition for preventive medicine

Improved stratification of CIMT-related risk is important for secondary prevention in the general population. Tosetto et al.[32] argued for age-adjusted CIMT reference limits to better predict cardiovascular risk by associating CIMT with the Framingham risk score. Eikendal et al.[11] have found a significant linear CIMT-risk relation with relatively high HR of 1.40 (95% CI 1.11-1.76) per standard deviation for CVEs in adults under 45 years in a study population of more than 3000 participants.

Both studies show that age stratification of CIMT provides some improvement to characterise CVE risk. However, the complication of attained age being a confounder to CIMT is not fully relieved. Both covariables exhibit strong positive correlation with each other and with CVE as outcome. To separate the age influence on CVE risk imparted by CIMT we introduced an auxiliary covariable dnCIMT, which is defined as the difference of CIMT to its sex and age-adjusted normal value. Comparison of Figs. 2 and S5 suggests that the decomposition of CIMT into dnCIMT and normCIMT can reveal nonlinear relationships with CVE risk which have hitherto been masked by confounding age. Further studies in other pertinent cohorts are encouraged to confirm the added value of introducing dnCIMT as risk factor. In this context, the relevance of a negative slope for the risk response at negative dnCIMT, which could reflect low stability of thin arterial walls, should be clarified (Fig. 2).

In the generic example of Supplementary Fig. 7 the conventional linear model involving CIMT and the logistic step model involving dnCIMT mostly identify the same F4 participants with elevated risk HR > 1.2. However, the mean age of participants with elevated risk identified only by the logistic model is much younger (45 yr) compared to the linear model (> 70 yr). The identification of otherwise asymptomatic patients with elevated CVE risk at younger age facilitates more effective preventive care.

Financial support

This project has received funding from the EURATOM research and training programme 2014-2018 under grant agreement No 755523 (MEDIRAD).

The KORA study was initiated and financed by the Helmholtz Zentrum München – German Research Centre for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria.

Measurement of CIMT was partly funded by grants of the Karl-Wilder-Foundation (JS) and the Deutsche Diabetes-Gesellschaft (CT).

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

JCK conceived the study, analysed the data and edited the manuscript, CS analysed the data and wrote the manuscript, MH was substantially involved in data collection, preparation and quality control and revised the manuscript for important scientific content, SR provided input regarding the statistical methods and revised the manuscript for important scientific content, CT and JS participated in the design of theC IMT study and performed CIMT measurements, AP designed the KORA study, handled findings and oversaw quality assurance measures.

References

[1] Libby, P, Inflammation in atherosclerosis, Arterioscler Thromb Vasc Biol, 2012;32:2045-2051.

[2] Ebrahimi, AP, Mechanical properties of normal and diseased cerebrovascular system, J Vasc Interv Neurol, 2009;2:155-162.

[3] Shadwick, RE, Mechanical design in arteries, J Exp Biol, 1999;202:3305-3313.

[4] Bentzon, JF, Otsuka, F, Virmani, R, et al., Mechanisms of plaque formation and rupture, Circ Res, 2014;114:1852-1866.

[5] Bauer, M, Caviezel, S, Teynor, A, et al., Carotid intima-media thickness as a biomarker of subclinical atherosclerosis, Swiss Med Wkly, 2012;142:w13705.

[6] Bauer, M, Mohlenkamp, S, Lehmann, N, et al., The effect of age and risk factors on coronary and carotid artery atherosclerotic burden in males-Results of the Heinz Nixdorf Recall Study, Atherosclerosis, 2009;205:595-602.

[7] Stein, JH, Korcarz, CE, Hurst, RT, et al., Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine, J Am Soc Echocardiogr, 2008;21:93-111; quiz 189-190.

[8] Lorenz, MW, Markus, HS, Bots, ML, et al., Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis, Circulation, 2007;115:459-467.

[9] Nambi, V, Chambless, L, Folsom, AR, et al., Carotid intima-media thickness and presence or absence of plaque improves prediction of coronary heart disease risk: the ARIC (Atherosclerosis Risk In Communities) study, J Am Coll Cardiol, 2010;55:1600-1607.

[10] Dawson, JD, Sonka, M, Blecha, MB, et al., Risk factors associated with aortic and carotid intima-media thickness in adolescents and young adults: the Muscatine Offspring Study, J Am Coll Cardiol, 2009;53:2273-2279.

[11] Eikendal, AL, Groenewegen, KA, Anderson, TJ, et al., Common carotid intima-media thickness relates to cardiovascular events in adults aged <45 years, Hypertension, 2015;65:707-713.

[12] Dinenno, FA, Jones, PP, Seals, DR, et al., Age-associated arterial wall thickening is related to elevations in sympathetic activity in healthy humans, Am J Physiol Heart Circ Physiol, 2000;278:H1205-1210.

[13] Tanaka, H, Dinenno, FA, Monahan, KD, et al., Carotid artery wall hypertrophy with age is related to local systolic blood pressure in healthy men, Arterioscler Thromb Vasc Biol, 2001;21:82-87.

[14] Bots, ML, Hofman, A and Grobbee, DE, Increased common carotid intima-media thickness. Adaptive response or a reflection of atherosclerosis? Findings from the Rotterdam Study, Stroke, 1997;28:2442-2447.

[15] Rathmann, W, Strassburger, K, Heier, M, et al., Incidence of Type 2 diabetes in the elderly German population and the effect of clinical and lifestyle risk factors: KORA S4/F4 cohort study, Diabet Med, 2009;26:1212-1219.

[16] Meisinger, C, Ruckert, IM, Rathmann, W, et al., Retinol-binding protein 4 is associated with prediabetes in adults from the general population: the Cooperative Health Research in the Region of Augsburg (KORA) F4 Study, Diabetes Care, 2011;34:1648-1650.

[17] Lowel, H, Lewis, M, Hormann, A, et al., Case finding, data quality aspects and comparability of myocardial infarction registers: results of a south German register study, J Clin Epidemiol, 1991;44:249-260.

[18] Bothig, S, WHO MONICA Project: objectives and design, Int J Epidemiol, 1989;18:S29-37.

[19] Alpert, JS, Thygesen, K, Antman, E, et al., Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction, J Am Coll Cardiol, 2000;36:959-969.

[20] Luepker, RV, Apple, FS, Christenson, RH, et al., Case definitions for acute coronary heart disease in epidemiology and clinical research studies: a statement from the AHA Council on Epidemiology and Prevention; AHA Statistics Committee; World Heart Federation Council on

Epidemiology and Prevention; the European Society of Cardiology Working Group on Epidemiology and Prevention; Centers for Disease Control and Prevention; and the National Heart, Lung, and Blood Institute, Circulation, 2003;108:2543-2549.
[21] Machon, M, Arriola, L, Larranaga, N, et al., Validity of self-reported prevalent cases of stroke and acute myocardial infarction in the Spanish cohort of the EPIC study, J Epidemiol Community Health, 2013;67:71-75.
[22] Kowall, B, Ebert, N, Then, C, et al., Associations between blood glucose and carotid intima-media thickness disappear after adjustment for shared risk factors: the KORA F4 study, PLoS One, 2012;7:e52590.
[23] Then, C, Kowall, B, Lechner, A, et al., Plasma MR-proANP levels are associated with carotid intima-media thickness in the general community: the KORA F4 study, Atherosclerosis, 2013;230:235-241.
[24] Bots, ML, Mulder, PG, Hofman, A, et al., Reproducibility of carotid vessel wall thickness measurements. The Rotterdam Study, J Clin Epidemiol, 1994;47:921-930.
[25] Mühlberger, N, Behrend, C and Stark, R, Datenbankgestützte Online-Erfassung von Arzneimitteln im Rahmen gesundheitswissenschaftlicher Studien - Erfahrungen mit der IDOM-Software., Informatik Biometrie Epidemiologie Medizin Biologie, 2003;34:601 - 611.
[26] Moore, DF, Applied Survival Analysis Using R, Springer International Publishing, 2016.
[27] Team, RC, R: A Language and Environment for Statistical Computing, In, Vienna, Austria, R Foundation for Statistical Computing, 2017.
[28] Chambless, LE, Folsom, AR, Clegg, LX, et al., Carotid wall thickness is predictive of incident clinical stroke: the Atherosclerosis Risk in Communities (ARIC) study, Am J Epidemiol, 2000;151:478-487.
[29] Simonetto, C, Azizova, TV, Barjaktarovic, Z, et al., A mechanistic model for atherosclerosis and its application to the cohort of Mayak workers, PLoS One, 2017;12:e0175386.
[30] Fernandez-Alvarez, V, Lopez, F, Suarez, C, et al., Radiation-induced carotid artery lesions, Strahlenther Onkol, 2018.
[31] Wilbers, J, Dorresteijn, LD, Haast, R, et al., Progression of carotid intima media thickness after radiotherapy: a long-term prospective cohort study, Radiother Oncol, 2014;113:359-363.
[32] Tosetto, A, Prati, P, Baracchini, C, et al., Age-adjusted reference limits for carotid intima-media thickness as better indicator of vascular risk: population-based estimates from the VITA project, J Thromb Haemost, 2005;3:1224-1230.

Tables

Table 1. Characteristics of the KORA F4 analysis sample (n=2580), p-values from hypothesis testing on differences in covariables for subjects with and without cardiovascular events (CVEs).

Covariable	Total (n=2580)	No CVE (n=2430)	CVE (n=153)	*p*-value
[a]Age at examination (AaE), (yr)	55.1 ± 12.9	54.5 ± 12.8	63.6 ± 10.8	< 0.001[d]
[b]Women	1351 (52)	1302 (54)	49 (32)	< 0.001[e]
[a]Body mass index (BMI) (kg/m^2)	27.6 ± 4.7	27.5 ± 4.7	29.2 ± 4.2	< 0.001[d]
[c]Alcohol consumption (AC) (g/day)	5.71 [0, 20.0]	5.71 [0, 40.0]	6.60 [0, 22.9]	0.73[d]
[b,g]Hypertension (HT+)	943 (37)	835 (34)	108 (71)	< 0.001[e]
[b,h]Dyslipidaemia (DL+)	510 (20)	462 (19)	48 (31)	0.0003[e]
[b,i]Statin intake (ST+)	227 (9)	209 (9)	18 (12)	0.23[e]
[b,j]Smoking (never smokers)	1109 (43)	1058 (44)	51 (33)	
[b,j]Smoking (light smokers)	569 (22)	546 (22)	23 (15)	
[b,j]Smoking (heavy smokers)	902 (35)	823 (34)	79 (54)	< 0.001[e]
[a]Carotis intima media thickness (CIMT) (mm)	0.847 ± 0.139	0.841 ± 0.136	0.940 ± 0.147	< 0.001[f]
[a]Deviation from age and sex-adjusted normCIMT (dnCIMT) (µm)	15.6 ± 102	13.6 ± 100	47.7 ± 129	0.0015[f]

[a]Mean ± standard deviation, [b]no. of subjects (%), [c]median [Q1, Q3]

[d]Mann-Whitney test, [e]X^2 test, [f]t test

[g]HT+: blood pressure of 140/90 mmHg or higher, or use of antihypertensive medication

[h]DL+: ratio of total cholesterol/HDL cholesterol 5 or higher

[i]ST+: categorised according to the Anatomical Therapeutical Chemical (ATC) classification index

[j]Light smokers: 10 pack years or less, heavy smokers: more than 10 pack years, pack contains 20 cigarettes

Table 2. Estimates for hazard ratios (HRs) (95% CI in brackets), p-values and AICs from Cox regression for models characterised by linear or logistic step dependence of ln(HR) on vascular covariables CIMT or dnCIMT. Supplementary Tables 3-6 and 8 show HR estimates for *all* available covariables.

Model	**Adjusting covariables**	**HR of vascular covariable (95% CI)**	**HR unit**	***p*-value**	**AIC**
Baseline (without vascular covariable)	sex, [a]age				2183.1
	all available				2146.8
Models involving CIMT as vascular covariable					
Linear	sex, [a]age	8.49 (2.21 – 32.6)	per mm	0.0018	2175.5
	all available	4.78 (1.20 – 19.1)	per mm	0.027	2143.9
Logistic step (centre at CIMT = 1.01 mm fitted, slope 0.02 mm fixed)	sex, [a]age	2.08 (1.40 - 3.08)	-	0.0003	2174.1
	all available	1.81 (1.23 - 2.68)	-	0.0028	2142.1
Models involving dnCIMT as vascular covariable					
Linear	sex, [a]age	8.63 (2.25 – 33.1)	per mm	0.0017	2175.4
	all available	4.87 (1.22 – 19.4)	per mm	0.025	2143.8
Logistic step (centre at dnCIMT = 0.09 mm fitted, slope at 0.02 mm fixed)	sex, [a]age	2.25 (1.58 – 3.19)	-	< 0.0001	2167.7
	all available	1.93 (1.35 – 2.76)	-	0.0003	2138.2

[a]Age at examination (AaE)

Figures and Legends

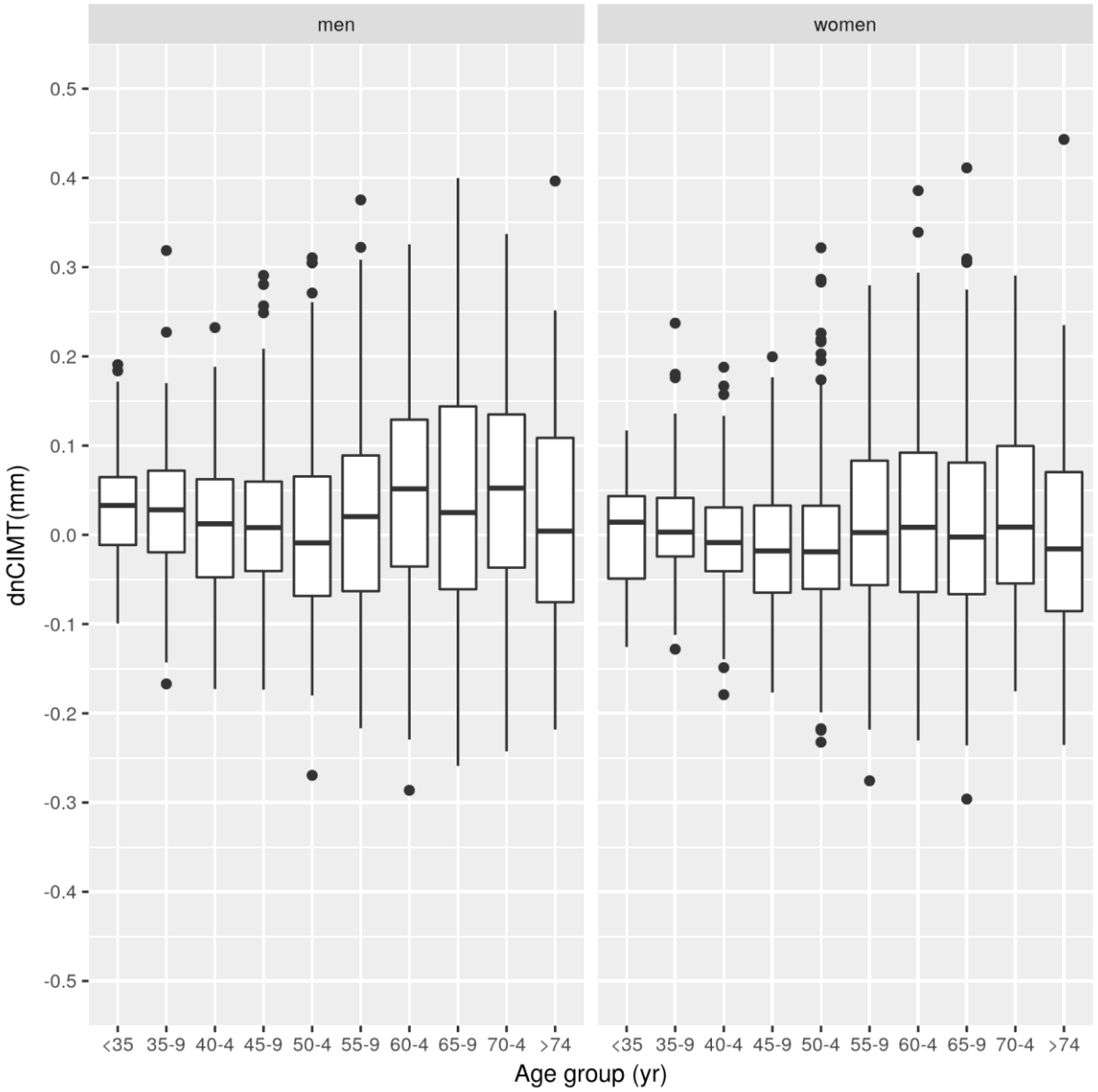

Fig. 1. Boxplots of sex-specific vascular covariable dnCIMT = CIMT – normCIMT in 10 groups of age at examination.

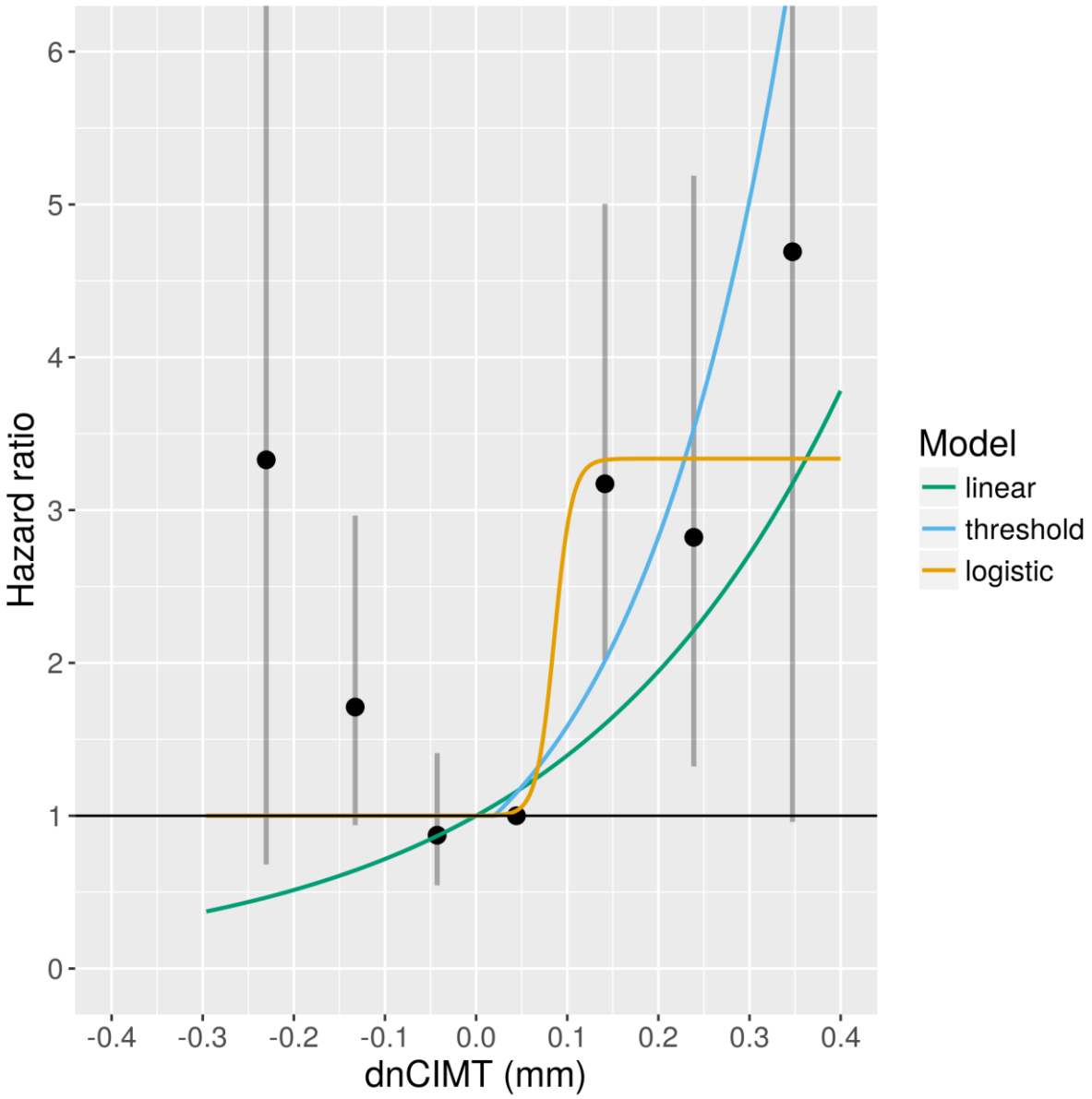

Fig. 2. Hazard ratios (HRs) from univariate Cox regression with vascular covariable dnCIMT = CIMT – normCIMT.

AIC = 2270.1 for the linear model is used as reference, for the threshold model the difference ΔAIC to the reference is - 11.9 and for logistic step model ΔAIC is -21.1; crude HRs (full points) with 95% CIs are shown for 7 intervals of dnCIMT values separated by 0.1 mm starting from -0.3 mm with reference HR = 1 for the centre interval 0 – 0.1 mm.

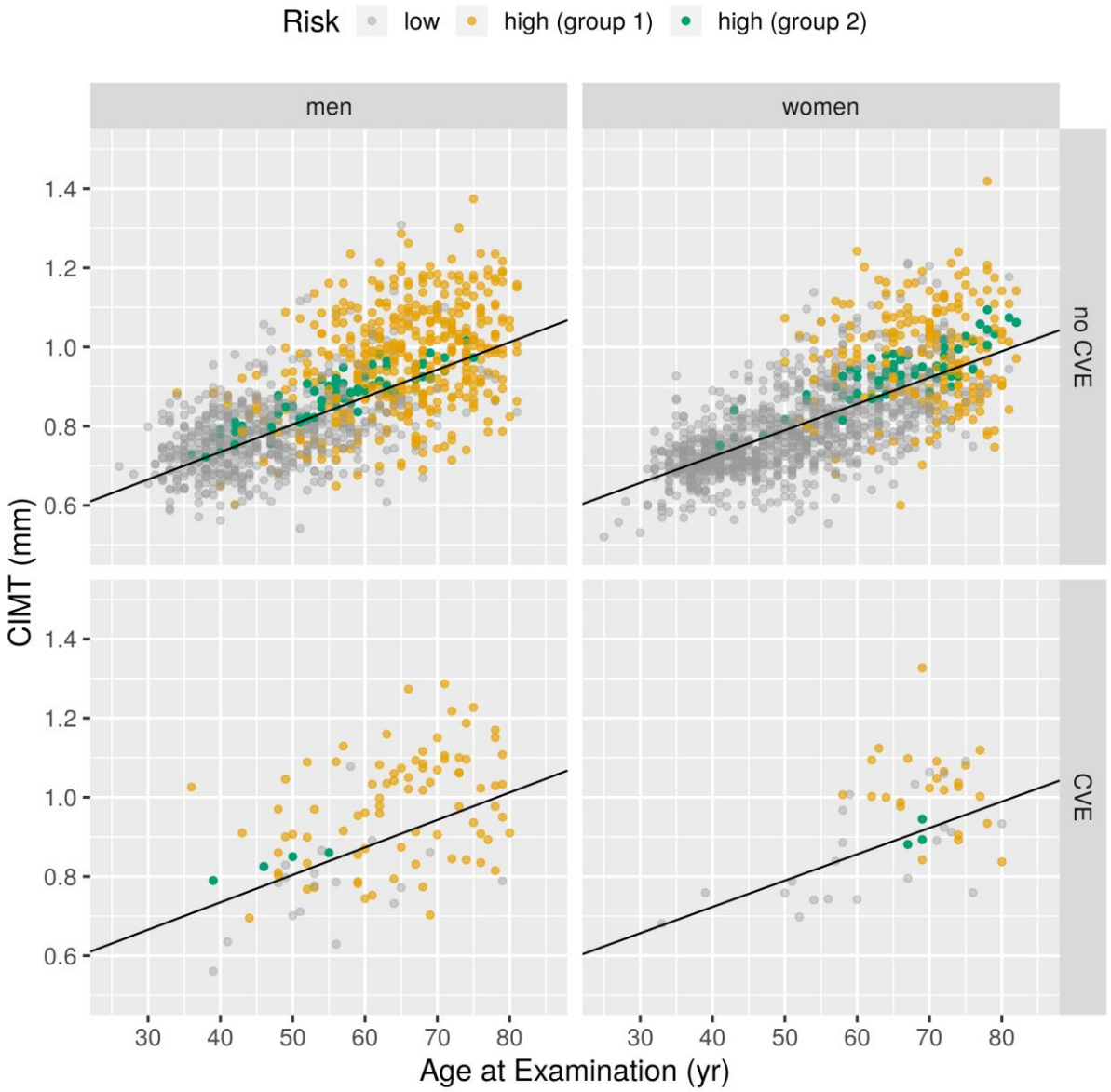

Fig. 3. Risk profile for KORA F4 2580 participants.

High risk (HR > 2) is defined in comparison to reference risk (HR = 1) according to the logistic step model involving dnCIMT and all other covariables (Table S8), 626 (18) men and 1021 (22) women without (with) CVEs exhibit a low risk profile; a high risk profile is shown for two disjunct groups, in *group 1* 428 (82) men and 212 (24) women without (with) CVEs possess a high risk under the logistic model anyway, in *group 2* 71 (4) men and 69 (3) women without (with) CVEs would be additionally shifted into the high risk group if their dnCIMT increased by 0.1 mm (i.e. after radiotherapy).