INFLAMMATION, CALCIFICATION, AND RISK PREDICTION IN PATIENTS WITH STABLE CARDIOVASCULAR DISEASE

Beyond the scope of usual care

Cilie C. van 't Klooster

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Inflammation, calcification, and risk prediction in patients with stable cardiovascular disease
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INFLAMMATION, CALCIFICATION, AND RISK PREDICTION IN PATIENTS WITH STABLE CARDIOVASCULAR DISEASE

Beyond the scope of usual care

Inflammatie, calcificatie en risicopredictie in patiënten met bestaande hart- en vaatziekte

Buiten het kader van de reguliere zorg

(met een samenvatting in het Nederlands)

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Chapter 1

General introduction

General introduction

Cardiovascular disease and cancer, the two leading non-communicable diseases worldwide,[1,2] account for 31% and 16% of all global deaths respectively.[2] For several years, cardiovascular mortality rates have declined substantially due to improved detection and interventions of acute cardiovascular events and due to advanced preventive treatment strategies.[1,3] Although the trends in cardiovascular mortality have plateaued in recent years[4] and cardiovascular disease is still the number one cause of death globally, a transition in the predominant causes of death in the middle-aged is observed in which mortality from cancer will apparently become the leading cause of death.[5] In fact, in some high and upper middle income countries cancer mortality has already outranked fatal cardiovascular disease.[5,6]

The number of patients with established cardiovascular disease in a chronic phase is growing as a consequence of several factors, including the increased survival of patients with an acute manifestation of cardiovascular disease, population growth and ageing, and lifestyle habits such as sedentary behavior and obesity.[1,4,7] Globally, a number of 422.7 million prevalent cases of cardiovascular disease in 2015 was estimated.[4] Recent evaluations estimate the number of patients with some form of established cardiovascular disease in the United States on 24.3 million adults (9% of total adult population).[8] In the Netherlands, 1.55 million patients had chronic cardiovascular disease (also including congenital heart disease) in 2018,[7,9] with an expected number of 1.9 million in 2030.[9] Patients with established cardiovascular disease are at risk of recurrent cardiovascular disease, and are generally classified as very high risk (≥10% risk of fatal cardiovascular event within 10 years) according to guidelines.[10] Preventing second cardiovascular events in these patients is needed from a patient's, as well as an economic[11] perspective.

Part I Low-grade systemic inflammation

Cardiovascular disease and cancer; two pieces of the same puzzle?

In addition to the risk of recurrent cardiovascular disease, patients with established cardiovascular disease are also at a higher risk for cancer compared to the general population[12-15] (standardized incidence ratio of 1.19; 95%CI 1.10-1.29 adjusted for age, sex and calendar year[14]). Especially cancer of the respiratory tract, bladder, colorectum, and kidney are more common in patients with cardiovascular disease.[14] Although generally regarded as two separate entities, increasing evidence shows an overlap in risk factors for cardiovascular disease and cancer, suggesting common pathways of etiology and progression of disease.[16-18] Shared risk factors for cardiovascular disease and cancer include lifestyle habits such as smoking, diet, and physical activity, diabetes mellitus, obesity, and hypertension.[16] Underlying proposed pathophysiological pathways leading from these well-established cardiovascular risk factors to cancer are several. One of the mechanisms through which diabetes and obesity are related to the development of certain malignancies, is chronic hyperinsulinemia and consequent elevated unbounded insulin-like growth factor (IGF-1) levels, resulting in promotion of cell proliferation.[12,16,18] Hypertension in turn, is associated with elevated levels of plasma vascular endothelial growth factor, a hormone that enables tumor cells to induce new blood-vessel formation.[16] Most importantly, inflammation is thought be one of the major common pathways leading from several risk factors such as diabetes mellitus, obesity, hypertension, and lifestyle habits to development and progression of both cardiovascular disease and cancer.[16]

Inflammation, cardiovascular disease, and cancer

Chronic low-grade systemic inflammation is a well established risk factor for cardiovascular disease, and plays a role in the etiology and progression of disease by initiating and accelerating arterial plaque formation and destabilization, causing acute atherosclerotic events.[19] The interleukin (IL) 1β, IL-6, C-reactive protein (CRP) pathway is involved in the pathogenesis; cholesterol crystals, neutrophil extracellular traps, atheroprone flow, and local tissue hypoxia activate the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome resulting in production of pro-IL1β and IL1β.[20] Moving downstream, IL-1β leads to IL-6 activation and consequent CRP production by the liver.[20] IL-6 signaling has been linked to plaque initiation and transformation to vulnerable plaques,[20-22] and microvascular flow dysfunction.[20,23]

The involvement of the IL1β, IL-6, CRP pathway in the pathophysiology of atherothrombosis is illustrated by the combined results of two trials; the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) and the Cardiovascular Inflammation Reduction Trial (CIRT), both enrolling patients with established cardiovascular disease.[20,24] In the CANTOS trial, canakinumab, an IL1β inhibitor, reduced CRP levels by 26-41% as well as recurrent cardiovascular disease incidence with a hazard ratio (HR) of 0.85 (95%CI 0.74-0.98) (150 mg canakinumab),[25] whereas methotrexate in CIRT had no influence on CRP levels nor incident cardiovascular disease.[24]

Another finding of the CANTOS trial was the reduced incidence of lung cancer, lung cancer death, and total cancer mortality in the treatment arm with HRs for the 300 mg canakinumab treatment arm of 0.33 (95%CI 0.18-0.59), 0.23 (95%CI 0.10-0.54), 0.49 (95%CI 0.31-0.75) respectively.[26] This finding supports the involvement of that same inflammatory pathway in (lung) cancer development and progression. Inflammation and cancer have been connected since 1863, when Rudolph Virchow noticed leucocytes in neoplastic tissues and linked chronic inflammation to cancer,[27] however, exact pathophysiological mechanisms are yet unclear. Hypothesized mechanisms of the role of inflammation in the development of cancer are focused on the promotion phase, and include stimulation of angiogenesis, vascular permeability, tumour cell survival and proliferation, and promotion of metastatic spread induced by IL-1β signaling pathways.[27-30]

In both cardiovascular disease and cancer, inflammatory markers upstream of CRP are believed causally related to development and progression of disease. Although it is unlikely that CRP itself is a chain in pathophysiological pathways leading to atherosclerotic disease and cancer,[20,31] CRP is a useful and stable downstream biomarker of systemic low-grade inflammation,[20] and as such can be implemented in etiologic as well as prognostic studies.

Part II Cardiovascular calcification

Cardiovascular calcification and recurrent cardiovascular disease

Whereas CRP is a marker of systemic inflammation, cardiovascular calcification correlates well with plaque amount, and can be considered a measure of total atherosclerotic burden.[32] Therefore, cardiovascular calcification scores are well suited for etiologic studies of atherosclerosis, as well as for prognostic studies of cardiovascular disease. Cardiovascular calcification is a complicated and multifaceted process[33] that varies between valvular[34,35] and vascular tissues. Even in vascular calcification there is a clear distinction in intimal and medial calcification.[36] Pathology is not yet completely understood, and questions remain as to why calcification develops in certain anatomical locations in one patient and different locations in another patient. Although similar pathways exist in calcification at various anatomical locations, including differentiation of the resident cell population to osteoblast-like bone producing cells and the loss of calcification inhibitors[33-35,37] leading to ectopic bone formation,[33-35] the impact of risk factors on initiation and progression of the calcification process differs,[34-38] potentially providing clues to remaining questions on variation in affected anatomical locations.

As a measure of total plaque burden, calcification scores are related to incident cardiovascular events[39-42] and calcification scores of coronary arteries were shown to improve risk reclassification in apparently healthy people[43,44] with increases in c-statistics ranging from 0.05 to 0.13 and reported net reclassification index (NRI) ranging from 14 to 25%.[43] In patients with established cardiovascular disease it is yet unclear whether cardiovascular calcification scores enhance risk prediction accuracy of recurrent cardiovascular events.

Part III Individualized risk prediction

Estimating individual risk of recurrent cardiovascular disease and cancer

Individualized cardiovascular risk prediction has become a part of patient care in daily clinical practice. Individual risk predictions provide information on prognosis for patients as well as clinicians and facilitate shared decision making by providing estimations of benefit from cardiovascular preventive treatment in comparison to potential treatment harms. Furthermore, risk predictions will identify those patients at the highest risk, potentially initiating more active screening and triggering lifestyle behavioral changes of patients, thereby potentially preventing (severe) disease. Although patients with established cardiovascular disease are, on average, regarded as high to very high risk by preventive treatment guidelines,[10] absolute 10-year risk of recurrent cardiovascular events varies in individual patients from <10% in 18% to >30% in 22% of the patients,[45] emphasizing the clinical use of risk prediction in patients with established cardiovascular disease.

Even though risk models are available for prediction of recurrent cardiovascular events in patients with established cardiovascular disease, with good calibration and moderate c-statistics varying from 0.62 to 0.68,[45-48] further improvement is constantly warranted. Improvement of risk estimations can be attained by managing various aspects of risk prediction, for example the addition of predictors, such as calcification scores, that could have a potential prognostic value in addition to traditional risk factors. Adjustment for temporal changes in the predicted outcome could be another aspect. Existing risk scores for patients with established cardiovascular disease are available to predict the risk of recurrent cardiovascular events.[46,48] However, incidence rates of recurrent cardiovascular disease have declined substantially over the last decades by 53% between 1996 and 2014 in a Dutch cohort of patients with stable cardiovascular disease.[49] Figure 1 illustrates the decline in recurrent major cardiovascular events in patients with established cardiovascular disease. Since the decline in recurrent cardiovascular events is only for 36% explained by changes in risk factors, medication use and subclinical atherosclerosis,[49] it is possible that due to earlier detection and improved percutaneous and surgical techniques, cardiovascular interventions have replaced part of the acute events. This trend indicates that it could also be clinically relevant to predict recurrent cardiovascular events and vascular interventions combined in patients with established cardiovascular disease.

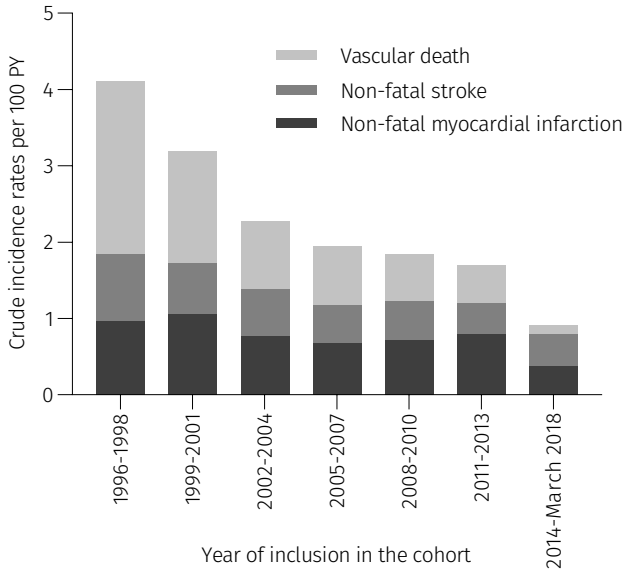

Figure 1. Temporal trend of incidence rates for recurrent cardiovascular events in a cohort of patients with established cardiovascular disease

Data from patients with established cardiovascular disease from the 'Utrecht Cardiovascular Cohort-Second Manifestations of ARTerial disease' (UCC-SMART) cohort (cohort description published elsewhere50). PY = person-years.

Furthermore, by acknowledging the higher risk of cancer in patients with established cardiovascular disease, it follows that it could also be clinically relevant to predict the risk of cancer in this specific patient population, in order to emphasize healthy lifestyle changes and potentially lower thresholds for targeted diagnostics in those patients with the highest predicted risks. The recommended timeframe for the cancer risk predictions will be dependent on the clinical use; usually, risk of disease is estimated from a 5-year or 10-year perspective and these might be most relevant with regard to potential selection of patients for (intensified) cancer screening. However, these risk estimates may not identify patients who have a relatively low 10 year absolute risk, but a high cumulative lifetime risk of cancer,[51] for example young smokers. Lifetime predictions can be calculated by using age as the underlying time axis, and estimating the cumulative risk or event-free survival probability by means of a lifetable.[51] These lifetime risks might give a more representative estimation of cancer risk, especially for young patients. And it is specifically in these younger patients that subsequent lifestyle adjustments could have the most beneficial effect with regard to cancer prevention.

Lastly, the elevated risk of cancer in patients with stable cardiovascular disease stresses the importance of another aspect in risk prediction; the adjustment for competing events. Competing events are outcomes that prevent a disease from occurring, such as mortality from other causes than the disease of interest.[51] Not taking competing risks into account will cause overestimations of the cumulative incidence of the disease of interest.[51] Cardiovascular disease and cancer are the most common causes of death in patients with stable cardiovascular disease with 47% and 35% of total deaths at 10 year respectively.[52] By accounting for these when estimating the risk of either recurrent cardiovascular events or the risk of cancer, risk predictions could become more accurate, enabling the correct identification of patients at the highest risk.

Thus, in patients with established cardiovascular disease, the risk of both recurrent cardiovascular disease and cancer should be acknowledged. Further study of etiologies of both diseases could enhance knowledge and lead to new preventive treatment strategies, whereas developing and improving risk prediction models to enable accurate identification of patients at the highest risk could stimulate adequate responses in clinical practice, potentially contributing to reduction of the global burden of disease.

Objectives of this thesis

The objectives of this thesis are:

Part I Systemic low-grade inflammation

- To quantify the relation between systemic low-grade inflammation and cancer in patients with established cardiovascular disease (**chapter 2**)
- To evaluate the relation between lifestyle improvements and change in systemic low-grade inflammation in patients with established cardiovascular disease (**chapter 3**)

Part II Cardiovascular calcification

- To investigate multifocal cardiovascular calcification in patients with established cardiovascular disease, with regard to prevalence, association with traditional atherosclerotic risk factors, and relation with recurrent cardiovascular events and vascular interventions (**chapter 4**)
- To evaluate the potential added prognostic value of cardiovascular calcification scores in addition to traditional risk factors for the prediction of recurrent cardiovascular events and vascular interventions in patients with established cardiovascular disease (**chapter 5**)

Part III Individualized risk prediction

- To develop and externally validate prediction models for estimating the risk of recurrent cardiovascular events and vascular interventions combined, as well as the risk of cancer, in patients with established cardiovascular disease (**chapter 6** and **chapter 7**)

Outline of this thesis

Part I of this thesis focuses on **systemic low-grade inflammation** as a risk factor for cardiovascular disease and cancer in patients with manifest cardiovascular disease. In **chapter 2** systemic low-grade inflammation measured by CRP is investigated as a risk factor for recurrent cardiovascular disease and cancer in patients with manifest cardiovascular disease. In **chapter 3** the effects of lifestyle improvements; smoking cessation, weight loss, physical activity increase, and alcohol use moderation, on systemic low-grade inflammation, measured by CRP, are evaluated in patients with established cardiovascular disease.

Part II focuses on **cardiovascular calcification** in patients with stable cardiovascular disease. In **chapter 4**, calcification of coronary arteries, thoracic aorta, and mitral annulus and aortic valve is studied in patients with established cardiovascular disease. Prevalence, association of risk factors with calcification at the different anatomical locations, and relation of calcification scores with recurrent cardiovascular events and vascular interventions are described. In **chapter 5** the potential added prognostic value of cardiovascular calcification scores in addition to traditional cardiovascular risk factors is evaluated for the prediction of recurrent cardiovascular events and vascular interventions combined.

Part III of this thesis focuses on individualized **risk prediction** of recurrent cardiovascular disease and cancer in patients with manifest cardiovascular disease. In **chapter 6** a prediction model is developed and externally validated to estimate the risk of recurrent cardiovascular events and cardiovascular interventions combined in patients with established cardiovascular disease. In **chapter 7** risk prediction models are developed and externally validated to estimate the risk of total, colorectal, and lung cancer in patients with established cardiovascular disease.

The main findings of the studies mentioned above are discussed in **chapter 8**. A summary of the results is provided in **chapter 9**.

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Part I

Systemic low-grade inflammation

Chapter 2

The relation between systemic inflammation and incident cancer in patients with stable cardiovascular disease, a cohort study

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European Heart Journal. 2019 Dec 21;40(48):3901-3909

Abstract

Aims

Low-grade inflammation, measured by elevated plasma concentrations of high sensitive C-reactive Protein (CRP), is a risk factor for cardiovascular disease (CVD). There is evidence that low-grade inflammation is also related to a higher risk of cancer. The present prospective cohort study evaluates the relation between low-grade systemic inflammation and risk of cancer in patients with stable cardiovascular disease.

Methods and results

In total 7178 patients with stable cardiovascular disease and plasma CRP levels ≤10 mg/L were included. Data were linked to the Dutch national cancer registry. Cox regression models were fitted to study the relation between CRP and incident CVD and cancer. After a median follow-up time of 8.3 years (interquartile range 4.6-12.3) 1072 incident cancer diagnoses were observed. CRP concentration was related to total cancer (HR 1.35; 95% CI 1.10-1.65) comparing last quintile to first quintile of CRP. Especially lung cancer, independent of histopathological subtype, was related to CRP (HR 3.39; 95%CI 2.02-5.69 comparing last to first quintile of CRP). Incidence of epithelial neoplasms and especially squamous cell neoplasms were related to CRP concentration, irrespective of anatomical location. Sensitivity analyses after excluding patients with a cancer diagnosis within one, two, and five years follow-up showed similar results. No effect modification was observed by smoking status or time since smoking cessation (p-values for interaction >0.05).

Conclusion

Chronic systemic low-grade inflammation, measured by CRP levels ≤10 mg/L, is a risk factor for incident cancer, markedly lung cancer, in patients with stable cardiovascular disease. The relation between inflammation and incident cancer is seen in former and current smokers, and is uncertain in never smokers.

Introduction

Chronic systemic low-grade inflammation plays an important role in the aetiology of atherosclerotic disease by initiating and accelerating arterial plaque formation and transformation to vulnerable plaques.[1] Besides the role in atherosclerotic disease, there is evidence that low-grade inflammation is related to a higher risk of incident cancer; previous prospective cohort studies found an increased risk of incident cancer related to higher C-reactive Protein (CRP) levels in population based cohorts or in cohorts of apparently healthy people[2-10]. Especially a higher risk of lung cancer was observed, with hazard ratios of 2.2; 95%CI 1.0-4.6 [4] and 2.8; 95%CI 1.6-4.9 [5] for patients with plasma CRP concentrations >3 mg/L versus <1 mg/L. In the CANTOS trial, which randomized patients in the stable phase after myocardial infarction to placebo or canakinumab, lowering CRP with an interleukin (IL) 1β antibody lowered the incidence of cardiovascular disease (CVD)[11] as well as lung cancer, lung cancer death, and total cancer mortality.[12]

CRP is part of the IL-1β, IL-6 inflammatory cascade, and can serve as a marker of systemic low-grade inflammation.[13] It is unlikely that CRP itself is causally related to cancer development, as genetically elevated CRP is not related to risk of cancer in a Mendelian randomization study.[14] Postulated mechanisms for the role of low-grade inflammation in the development of cancer are focused on the promotion phase, and include stimulation of tumour cell survival and proliferation, and promotion of metastatic spread.[15,16] Chronic systemic low-grade inflammation, commonly defined as CRP levels ≤ 10mg/L,[17] is caused by various factors including smoking, abdominal obesity, atrial fibrillation or heart failure.[18] Shared risk factors for both CVD as well as cancer include smoking and (abdominal) obesity.[19] In turn, these risk factors increase levels of systemic low-grade inflammation, further suggesting that low-grade inflammation could be a common pathway leading to CVD and to cancer. Moreover, patients with stable CVD have a higher risk of cancer than the general population.[20] These patients could benefit from therapy directed at lowering inflammation to reduce recurrent CVD risk as well as cancer risk.[11,12]

In the present study the relation is evaluated between systemic low-grade inflammation and risk of recurrent CVD and incident cancer in patients with stable cardiovascular disease.

Methods

Study population

Patients originated from the Second Manifestations of ARTerial disease (UCC-SMART) cohort, an ongoing prospective cohort study since 1996, including 18-79 year-old patients referred to the University Medical Centre Utrecht (UMCU), the Netherlands. Central aim of the UCC-SMART cohort is to gain insight in arterial disease occurrence and risk factors for (recurrent) cardiovascular events. For the current study, patients with established cardiovascular disease at baseline between September 1996 and March 2017 were included (N=8139). Inclusion in the UCC-SMART cohort occurs at least two months after the qualifying vascular event. The institutional review board of the UMCU approved the study and all patients gave written informed consent. Patients who did not give permission for data requests to other medical authorities were excluded (N= 269). Study design and rationale have been described previously.[21] In short, information on medical history and lifestyle was acquired and physical examination measurements were obtained according to a standardized protocol. Metabolic syndrome was defined according to the National Cholesterol Education Program Adult Treatment Panel III definition.[22] High sensitive CRP level was determined by immunonephelometry (Nephelometer Analyzer BN II, Dade-Behring). From 2013 high sensitive CRP was determined in heparin plasma on an AU5811 routine chemistry analyzer (Beckman Coulter, Brea, California). Kidney function was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[23]

Follow- up

During follow-up participants received questionnaires biannually, gathering information on occurrence of recurrent CVD, bleeding events, incident diabetes and end stage renal disease. Additional information was gained by collecting hospital or general practitioner's data. Three physicians from the endpoint committee independently adjudicated all clinical events and conflicting classifications were discussed. The number of patients lost to follow-up was 412 (5.7%).

Data on cancer incidence and details of cancer types and histopathology were obtained by linking the UCC-SMART database to the Dutch National Cancer Registry (INKL), a national registry receiving notifications of all new cancer diagnoses in the Netherlands through the Nationwide Network and Registry of Histopathology and Cytopathology (PALGA), and hospital discharge diagnoses. For the current study, benign tumours, in situ neoplasms, non-melanoma skin cancer, and neoplasms of unknown or uncertain behaviour (eg. polycythemia vera) were excluded. Cancer diagnoses were classified according to anatomical location of origin and according to histopathology (Appendix 1 and 2).

Data preparation

Missing data for hsCRP level (n=97 (1.2%)), smoking (n=28 (0.3%)), pack-years (n=32 (0.4%)), body mass index (BMI) (n=18 (0.2%)), low density lipoprotein cholesterol (LDL-c) (n=138 (1.7%)), and systolic blood pressure (SBP) (n=18 (0.2%)), were imputed. Single imputation was performed using bootstrapping and predictive mean matching based on multivariable regression including independent variables and outcome data (aregImpute-function in R, Hmisc-package). As CRP levels >10 mg/L are commonly associated with an acute inflammatory response,[17] these patients (N=690) were excluded. Two patients had a recurrence of the same cancer diagnosed before entering the cohort and were therefore excluded.

Data analyses

Patients were stratified by quintiles of CRP level and baseline characteristics were displayed accordingly. Kaplan Meier survival curves were plotted per CRP quintile for recurrent CVD, CVD and/or cancer combined, total cancer, and lung cancer. Recurrent CVD was defined as the occurrence of myocardial infarction, stroke, or vascular death (Appendix 3).

Cox proportional hazard models were fitted to estimate hazard ratios (HR) with 95% confidence intervals (CI) describing the relation between CRP and recurrent CVD and incident cancer. With regard to the etiologic nature of the study, there was no need to take competing risks into account. Adjusted hazard ratios from Cox regression analyses were added to the Kaplan Meier plots. CRP was added to the model as a continuous and categorical variable. Subjects who were exempt from the outcome, were lost to follow-up or died of another cause were censored. Total cancer incidence was analysed, as well as cancer types separately, if a sufficient number of cases (>60) was present. Cancer types classified according to anatomical location of origin were taken as primary endpoint. Secondary outcome was cancer type classified according to histopathology. For the analyses of specific cancer types, the first diagnosis of that particular cancer was taken as the outcome, possibly being the second or third diagnosis of cancer during follow-up for a certain patient. Hazard ratios were adjusted for age and sex in model 1. Additionally, smoking status, pack-years of smoking, BMI, LDL-c, diabetes mellitus, SBP, and kidney function were considered potential confounders in the relation between CRP and CVD or cancer, and were added to model 2. Estimates did not change in exploratory models with addition of year of inclusion in the cohort, metabolic syndrome, or lipid-lowering or anti-platelet medication. To test potential effect modification by sex,[24] multiplicative interaction terms with CRP level were added to the models, showing no significant interactions (p-values >0.05).

Linearity assumption was tested visually by adding continuous CRP level as a restricted cubic spline function to the model. No violations were observed. The proportional hazards assumption, examined graphically by plotting scaled Schoenfeld residuals against time, was not violated.

Influence of BMI and smoking on the relation between CRP and cancer was evaluated. Multiplicative interaction terms with BMI and smoking status were added to the models to assess effect modification, and additional stratified analyses were performed for smoking status. Adjustment for BMI and smoking specifically was performed to evaluate mediation effects. For the relation between CRP and lung cancer, a multiplicative interaction term with time since smoking cessation was assessed, as well as additional adjustment for time since smoking cessation. To examine influence of CRP additional to smoking effects on (lung) cancer risk, analyses were performed with a categorical determinant combining smoking status with CRP quintile, using never smokers in the lowest CRP quintile as a reference group for total cancer. For lung cancer, due to the low event number in never smokers, former smokers in the lowest CRP quintile were taken as reference group.

To evaluate effect modification by interim non-fatal cardiovascular events, multiplicative interaction terms were added to models of total and lung cancer. Reverse causality was evaluated by repeating analyses after excluding patients diagnosed with cancer within one, two, and five year(s) after inclusion. Also, analyses were stratified for location of vascular disease (coronary artery disease, cerebrovascular disease, or peripheral vascular disease) at baseline. Additional sensitivity analyses were performed after exclusion of patients with any type of cancer (except non-melanoma skin cancer) before inclusion in the cohort, and after excluding patients with CRP levels >5 mg/L. Stability of CRP levels during follow-up was assessed in a subset of UCC-SMART patients who revisited for second measurements (N= 1794).

Results

In total 7178 patients with stable vascular disease and CRP levels ≤10 mg/L were included. Baseline characteristics stratified for CRP quintiles are shown in Table 1. Patients in the highest CRP stratum were more likely to be current smokers, generally had a higher number of pack-years, and fewer patients used lipid-lowering and antiplatelet medication. Other unfavourable trends with regard to cardiovascular risk profile in the highest CRP stratum included a slightly higher SBP, LDL-c, and higher prevalence of diabetes.

Relation between CRP and risk of recurrent cardiovascular events

During a median follow-up of 8.3 years (interquartile range (IQR) 4.6-12.3) and a total of 58,568 person-years of follow-up, 1289 patients experienced a recurrent cardiovascular event. Crude incidence rates were 1.53%, 1.55%, 2.07%, 2.64%, and 3.30% across CRP quintiles. Patients in the highest CRP quintile had a higher cardiovascular risk compared to patients in the lowest quintile of CRP (HR 1.58; 95%CI 1.31-1.91) (Figure 1A). The risk of cancer and/or CVD was 45% higher in the highest CRP quintile compared to the lowest (HR 1.45; 95%CI 1.26-1.68) (1B). CRP was significantly related to risk of myocardial infarction, vascular death, and all-cause mortality, but not to risk of stroke in categorical and continuous analyses (Appendix 4).

Relation between CRP and risk of incident cancer according to anatomical location of origin

During follow-up 1072 incident malignancies were observed. Most frequently occurring diagnoses were cancer of the lung (n= 226), prostate (188), and colon/rectum (n=177). Crude incidence rates per person-year were 1.53%, 1.49%, 1.65%, 2.01%, and 2.50% across CRP quintiles. Patients with a higher CRP level had a higher risk of cancer, comparing patients in the highest CRP quintile to patients in the lowest quintile (HR1.41; 95%CI 1.22-1.63) (Figure 1C and 2), and per 1 mg/L higher CRP (HR 1.07; 95%CI 1.04-1.09) (Figure 2). Risk of incident lung cancer was higher in the last CRP quintile compared to the first (HR 3.39; 95%CI 2.03-5.69) (Figure 1D and 2), and the risk increased 16% for each 1 mg/L higher CRP (HR 1.16; 95%CI 1.10-1.22) (Figure 2). Urinary tract cancer was possibly related to CRP concentration (HR 1.08; 95%CI 0.995-1.17 for every 1 mg/L higher CRP and HR 1.51; 95%CI 0.81-2.81 comparing last quintile with first CRP quintile) (Figure 2). Similarly, lymphoid/hematopoietic cancer was possibly related to CRP level, particularly in continuous analysis (HR 1.12; 95%CI 1.02-1.22 for every 1 mg/L higher CRP level, and HR1.65; 95%CI 0.81-3.35 comparing fifth quintile with first CRP quintile (Figure 2)). No relation was observed between CRP level and risk of breast or prostate cancer (in subgroups of women and men respectively), or incident colorectal cancer.

Table 1. Baseline characteristics stratified by quintiles of CRP level

	Quintile 1	**Quintile 2**
Median CRP (mg/L) (range)	0.50 (0.10-0.70)	1.00 (0.71-1.39)
n= 7178	n=1455	n=1417
Male	1129 (78%)	1075 (76%)
Age (years)*	58 ± 10	59 ± 10
Medical history		
Cancer (except non-melanoma skin cancer), n (%)	45 (3%)	55 (4%)
Cerebrovascular disease, n (%)	468 (32%)	404 (29%)
Coronary artery disease, n (%)	930 (64%)	945 (67%)
Peripheral artery disease, n (%)	145 (10%)	186 (13%)
Diabetes Mellitus, n (%)	202 (14%)	223 (16%)
Current smoking, n (%)	298 (20%)	333 (24%)
Former smoking, n (%)	703 (48%)	724 (51%)
Number of pack-years*	8 (0 - 23)	12 (0 - 27)
Metabolic syndrome, n (%)	520 (36%)	640 (45%)
Physical examination		
Body mass index (kg/m2)*	26 ± 3	26 ± 3
Waist circumference (cm)*	91 ± 11	94 ± 11
Systolic blood pressure (mmHg)*	136 ± 19	138 ± 20
Diastolic blood pressure (mmHg)*	80 ± 11	80 ± 11
Laboratory measurements		
Triglycerides (mmol/L)*	1.2 (0.9 - 1.6)	1.3 (1.0 - 1.9)
HDL-cholesterol (mmol/L)*	1.2 (1.0 - 1.4)	1.2 (1.0 - 1.4)
LDL-cholesterol (mmol/L)*	2.4 (1.9 - 3.1)	2.5 (2.0 - 3.3)
eGFR (CKD-EPI, mL/min/1.73m^2)*	80 ± 16	78 ± 17
Medication		
Lipid-lowering medication, n (%)	1123 (77%)	1070 (76%)
Blood pressure-lowering medication, n (%)	1052 (72%)	1081 (76%)
Anti-platelet therapy, n(%)	1190 (82%)	1158 (82%)
Anti-coagulants, n (%)	109 (7%)	133 (9%)

* Data are means ± SD for normal distributed data and median (interquartile range) for unevenly distributed data. CRP = high sensitive C-reactive protein.

	Quintile 3	Quintile 4	Quintile 5
	1.80 (1.39-2.30)	3.07 (2.31-4.10)	5.90 (4.10-10.00)
	n=1455	n=1426	n=1425
	1094 (75%)	1044 (73%)	1008 (71%)
	61 ± 10	61 ± 10	61 ± 10
	53 (4%)	79 (6%)	68 (5%)
	421 (29%)	431 (30%)	446 (31%)
	929 (64%)	868 (61%)	756 (53%)
	227 (16%)	298 (21%)	373 (26%)
	263 (18%)	248 (17%)	287 (20%)
	417 (29%)	519 (36%)	608 (43%)
	720 (49%)	662 (46%)	607 (43%)
	14 (3 - 31)	20 (6 - 35)	22 (9 - 37)
	796 (55%)	844 (60%)	910 (64%)
	27 ± 4	27 ± 4	28 ± 4
	97 ± 11	98 ± 12	98 ± 12
	140 ± 20	141 ± 20	142 ± 21
	81 ± 11	81 ± 11	82 ± 11
	1.4 (1.0 - 2.1)	1.5 (1.1 - 2.1)	1.6 (1.2 - 2.3)
	1.2 (1.0 - 1.4)	1.2 (1.0 - 1.4)	1.1 (0.9 - 1.3)
	2.7 (2.1 - 3.5)	2.8 (2.2 - 3.6)	3.0 (2.3 - 3.9)
	77 ± 17	76 ± 18	75 ± 20
	1021 (70%)	937 (66%)	843 (59%)
	1117 (77%)	1063 (75%)	1047 (73%)
	1138 (78%)	1078 (76%)	1004 (70%)
	177 (12%)	156 (11%)	184 (13%)

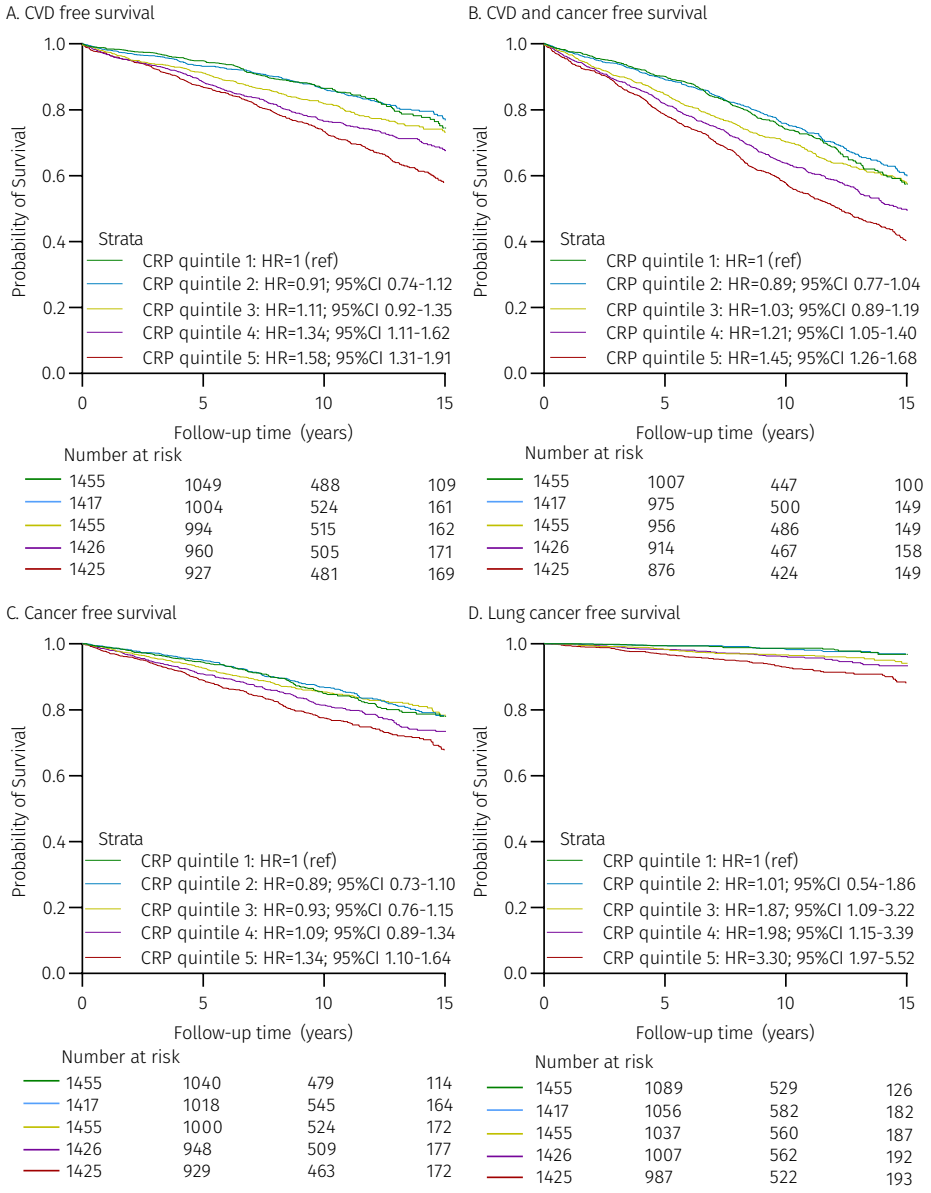

Figure 1. Survival curves in CRP quintiles for recurrent CVD, combined endpoint of recurrent CVD and cancer, total cancer, and lung cancer

Hazard ratios are adjusted for age, sex, body mass index, smoking status, pack-years of smoking, LDL cholesterol, diabetes mellitus, systolic blood pressure, and kidney function. Quintile 1: CRP 0.50 (range 0.10-0.70); Quintile 2: CRP 1.00 (range 0.70-1.39); Quintile 3: CRP 1.80 (range 1.39-2.30); Quintile 4: CRP 3.07 (range 2.31-4.10); Quintile 5: CRP 5.90 (range 4.10-10.00).

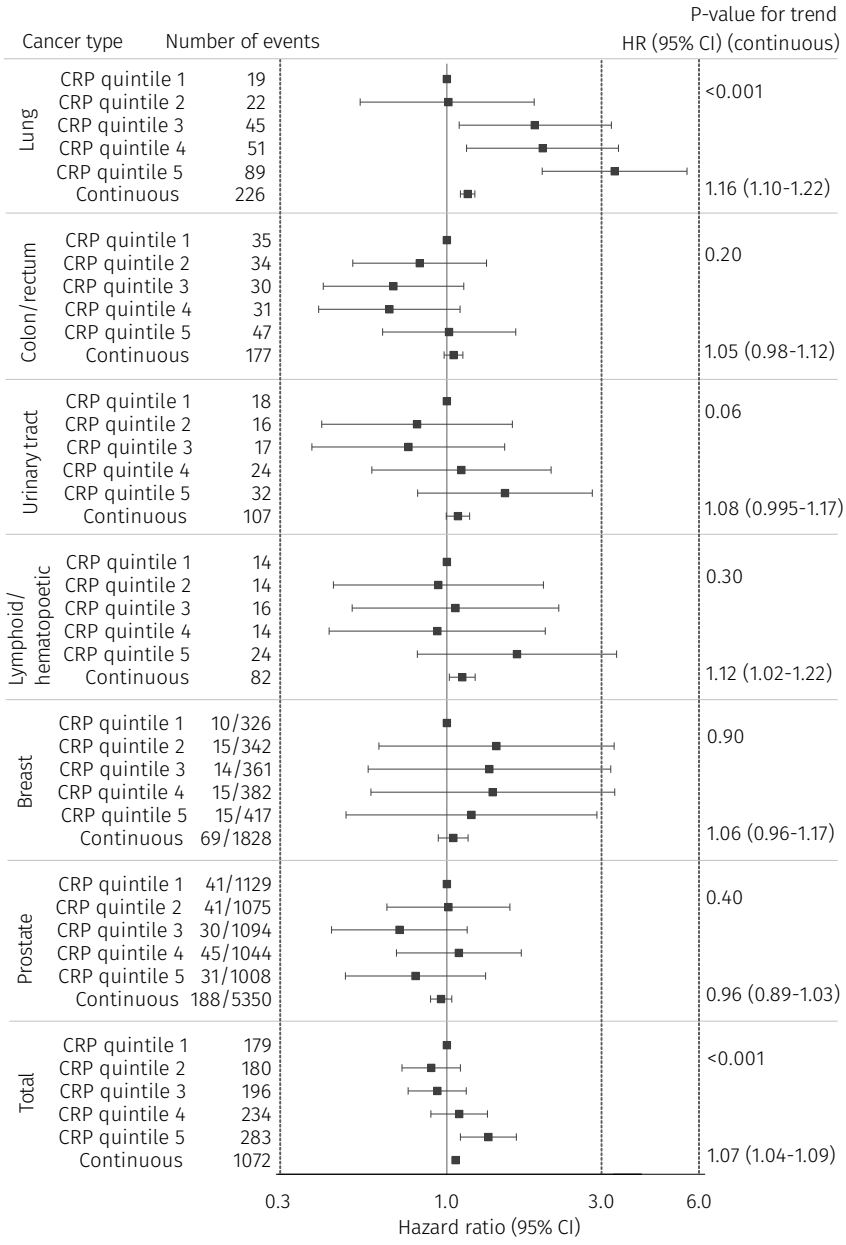

Figure 2. Relation between CRP and incident cancer, according to anatomical location of origin
Hazard ratios are adjusted for age, sex, smoking status, number of pack-years, body mass index, LDL cholesterol, diabetes mellitus, systolic blood pressure, and kidney function. Analyses for breast and prostate cancer were performed in subgroups of women and men respectively. Number of events per number of women or men in CRP quintiles are given. Continuous analyses represent hazard ratios per 1 mg/L higher CRP concentration.

Relation between CRP and risk of incident cancer according to histopathology

The relation between plasma CRP and risk of lung cancer was similar for histopathological subtypes; small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), including adenocarcinoma, squamous cell carcinoma, and large cell carcinoma (Appendix 5). In secondary outcome analyses with cancer types according to histopathology irrespective of anatomical location of origin, CRP was significantly related to risk of epithelial neoplasms not further specified (hereinafter referred to as epithelial neoplasms) (HR 1.17; 95%CI 1.08-1.27), and squamous cell neoplasms (HR 1.11; 95%CI 1.02-1.20) (Appendix 6).

Smoking and BMI, and the relation between CRP and risk of incident (lung) cancer

No significant interaction terms with BMI were observed (p-values >0.05). Adjustment for BMI or smoking did not mitigate the relation between CRP and cancer (Appendix 7). For the relation between CRP and lung cancer, no effect modification was observed by time since smoking cessation in former smokers (p-value for interaction 0.44) and additional adjustment for time since smoking cessation showed similar results (HR 1.17; 95%CI 1.11-1.23 compared to HR 1.16; 95%CI 1.10-1.22 of the original adjusted model). Stratified analyses for smoking status showed similar hazard ratios for lung and total cancer (p-values for interaction >0.05) (Appendix 8). Current smokers in the highest quintile of CRP had the highest risk of lung cancer (HR 11.70; 95%CI 4.95-27.64 compared to former smokers in the lowest CRP quintile) (p-value for trend <0.0001) (Figure 3A) and total cancer (HR 2.23; 95%CI 1.55-3.22 compared to never smokers in the lowest quintile) (p-value for trend <0.0001) (Figure 3B).

Sensitivity analyses

No significant interactions were observed with interim non-fatal CVD (p-values 0.72 and 0.33 for total cancer and lung cancer respectively). Reverse causality was evaluated by repeating analyses after excluding patients who were diagnosed with cancer within one year (n=102), two years (n=193), and five years (N=477) after entering the cohort, and showed similar results (Appendix 9). Analyses stratified for vascular disease location at baseline: coronary artery disease (n= 3931), cerebrovascular disease (n= 1904) or peripheral vascular disease (n= 1343) revealed similar results (Appendix 10). Similar results were observed after exclusion of patients with a history of cancer before inclusion (n=300), or after exclusion of patients with CRP levels >5mg/L (N=985) (Appendix 11 and 12). CRP levels were similar after a median of 9.9 years (IQR 5.4-10.8 years) with a mean difference of -0.18 mg/L (standard error of the mean 0.05).

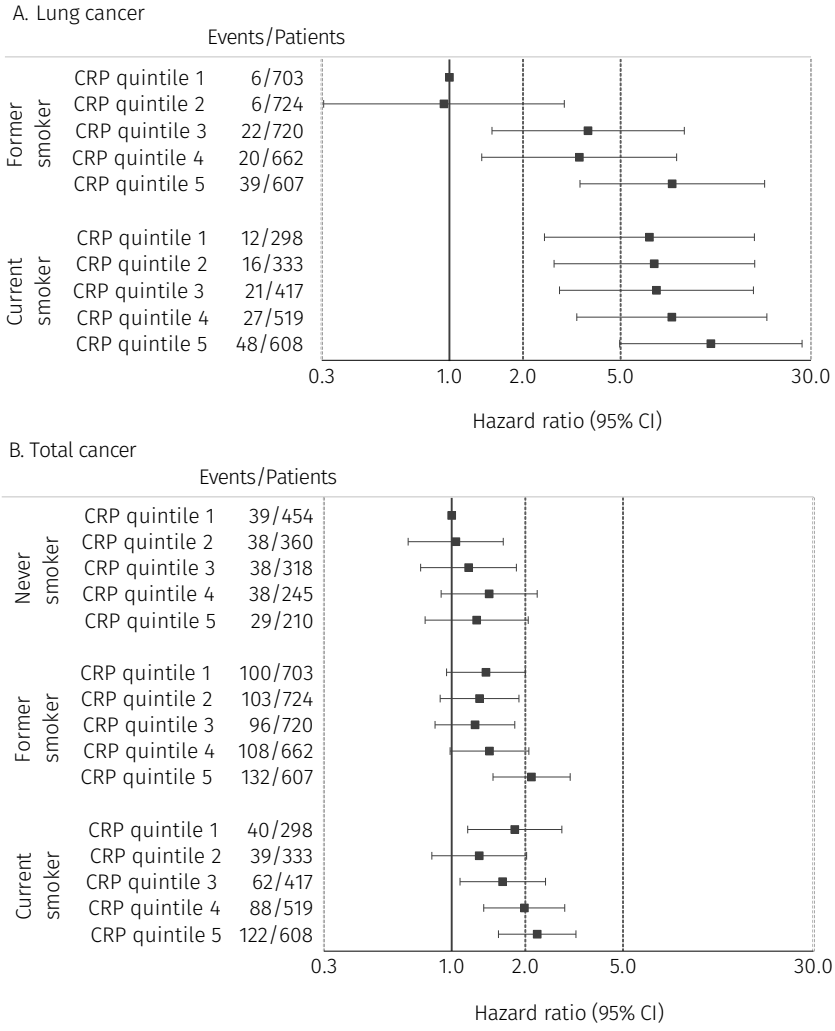

Figure 3. Relation between CRP quintiles with categories of smoking status and risk of cancer
Hazard ratios are adjusted for age, sex, body mass index, LDL cholesterol, diabetes mellitus, systolic blood pressure, and kidney function.

Discussion

The present study shows that in patients with stable vascular disease plasma CRP concentration is related to risk of recurrent cardiovascular events, as well as risk of cancer, especially lung cancer. No effect modification by smoking status was observed. A potential relation was observed between CRP and lymphoid/hematopoietic and urinary tract cancer. The relation between plasma CRP and incident cancer was seen for epithelial neoplasms, especially squamous cell neoplasms, irrespective of anatomical location of origin.

Results of the present study support the role of chronic systemic low-grade inflammation as a stimulating factor in cancer development in a cohort of patients with established vascular disease. The observed relation between CRP and cancer risk can not be explained by reverse causality, meaning that an elevated CRP would simply be a sign of occult cancer, as similar results were observed after exclusion of patients with a diagnosis of cancer within one, two, and five year(s) after inclusion. Results of the present study correspond to results of the CANTOS trial[11,12] and previous prospective cohort studies performed in population based cohorts or cohorts of apparently healthy people.[2-6,9,10] To our knowledge, no previous studies investigated the relation between CRP and incident cancer in patients with established vascular disease specifically. Cancer incidence is higher in patients with established CVD compared to the general population, likely due to common risk factors[20], and the current study shows that systemic low-grade inflammation is a contributing factor in pathophysiology of CVD as well as cancer.

In accordance with previous observational studies[2-6,9,25], and in line with the CANTOS trial results[12], lung cancer risk was especially related to CRP levels. Chronic low-grade inflammation is previously considered to be one of the causal pathways by which smoking leads to lung cancer.[15] Epithelial neoplasms and squamous cell neoplasms, irrespective of anatomical location of origin, were mostly respiratory tract cancers; lung carcinomas and carcinomas of the lip, oral cavity, pharynx, and glottis. The elevated systemic inflammatory levels as a risk factor for respiratory tract cancer might reflect a local inflammatory microenvironment caused by smoking[26] that contributes to cancer development. It is possible that low-grade inflammation initiated by smoking, is not reversed when quitting smoking, emphasizing the importance of smoking abstinence. In the present study, the relation between CRP and total cancer risk in never smokers was uncertain (HR 1.05; 95%CI 0.98-1.13). However, no significant interaction was observed for smoking status (p-values >0.05) and the point estimate was the same as in current smokers (HR 1.05; 95%CI 1.01-1.10). The incidence of lung cancer (n=9) in never smokers was too low for reliable analysis. A previous case-control study nested in population based cohorts showed no relation between CRP and lung cancer in never smokers.[25] However, that higher inflammation levels as a risk factor for cancer are a direct result of smoking is unlikely based on the results of this study. Adjustment for smoking status and pack-years did not mitigate the

relation between CRP level and cancer risk, suggesting that other pathophysiological pathways, and possibly other inflammatory pathways, play a role in mechanisms leading from smoking to cancer. Furthermore, the combination of a CRP level in the highest quintile with current smoking, conferred the highest cancer risk, suggesting an additive effect of inflammation and smoking on cancer risk. Potential relations between CRP and lymphoid/hematopoietic and urinary tract cancer should be interpreted with caution, as the relations were not statistically significant in all analyses, but suggest that inflammation could be involved in the pathogenesis of these neoplasms.

The relation between CRP and cancer risk is of great importance for clinical practice. As treatment for CVD has improved substantially over the last decades, more patients survive acute manifestations of cardiovascular disease and survive long enough to develop cancer. CRP is a marker for cardiovascular disease risk, and could potentially also serve as a prognostic marker to identify those at high risk of (lung) cancer. Since patients from the third CRP quintile and higher had an increased risk of lung cancer, CRP levels of ≥1.4 mg/L might be indicative of a higher risk of lung cancer. It could even be hypothesized that patients at high cardiovascular risk with high levels of inflammation are those that might benefit from anti-inflammatory treatment to reduce cardiovascular risk as well as risk of (lung) cancer. The CANTOS trial implicated that the interleukin-1 β, interleukin-6, CRP inflammatory pathway is involved in cancer development.[12] Results of trials studying other anti-inflammatory treatments could provide additional information on specific inflammatory pathways involved in cancer pathogenesis and the effectiveness of lowering inflammation on reduction of cancer risk, even though cancer was not the primary endpoint in these trials. However, the Cardiovascular Inflammation Reduction Trial (CIRT) was stopped due to ineffectiveness of methotrexate on CRP levels and CVD risk and no data is available yet on cancer incidence.[27] The Low Dose Colchicine study (LoDoCo2, EudraCT Number: 2015-005568-40), trialling effect of colchicine on CVD risk is still ongoing and might provide additional information.

Strengths of the present study include the large patient population with established vascular disease and the prospective study design with long follow-up, large number of events and histopathological cancer diagnoses. Potential limitations should be considered and include the single measurement of CRP level at baseline, as CRP levels might fluctuate during follow up. However, patients were included in the cohort at least two months after the qualifying cardiovascular event, thus stable on medication that might influence CRP levels. Moreover, repeated CRP measurements over time are shown to be stable in a subset of UCC-SMART patients with repeated measurement as well as previous research.[28] Data on other inflammatory markers, such as interleukin-6, was not available. Despite the large number of total cancer events, number of certain specific cancer types were insufficient for reliable analyses. Additionally, subgroups of smaller size with limited number of events, for example women, might be insufficient for

reliable subgroup analyses. The number of lung cancer cases in never smokers was insufficient for reliable analysis, and the relation between CRP and lung cancer can not be generalized to never smokers. Given the observational study design firm conclusions on causality should be made with caution as residual confounding cannot be ruled out.

Chronic systemic low-grade inflammation, measured by CRP levels ≤10 mg/L, is a risk factor for incident cancer, markedly lung cancer, in patients with stable cardiovascular disease. The relation between inflammation and incident cancer is seen in former and current smokers, and is uncertain in never smokers.

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Appendices

Appendix 1. Cancer diagnosis according to ICD-10 classification

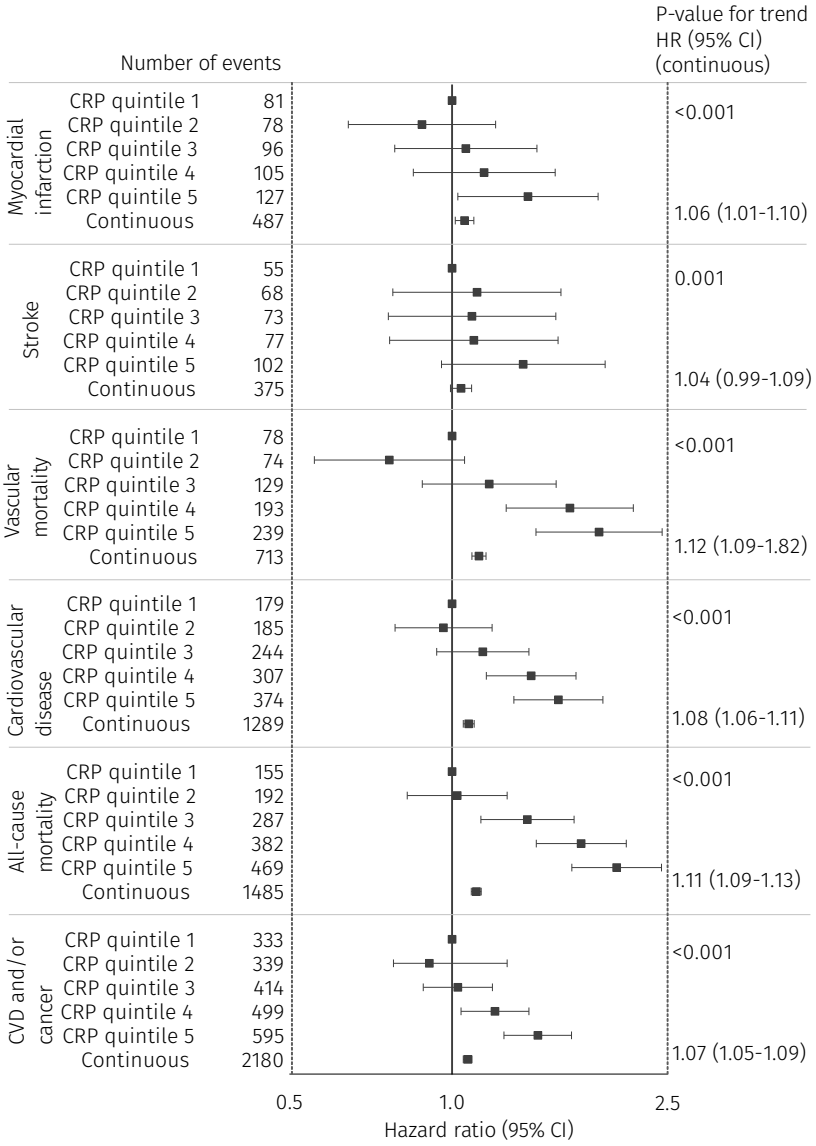
Cancer group	Topography	Number	ICD-10 code
Colon/rectum	Colon, rectum	177	C18-C20
Lung	Lung, bronchus	226	C34
Breast	Breast	70	C50
Prostate	Prostate	188	C61
Urinary tract	Kidney, renal pelvis, ureter	52	C64-C66
	Bladder, or unspecified parts of urinary organs	57	C67-C68
Lymphoid/ hematopoietic	Hodgkin's disease	1	C81
	Non-Hodgkin's lymphoma	30	C82-C85
	Multiple myeloma	19	C88, C90
	Leukemia	32	C91-C96
Melanoma skin cancer	Melanoma of skin	52	C43
Other	Lip, oral cavity, pharynx	31	C00-C14
	Esophagus	36	C15
	Stomach	38	C16
	Small intestine	6	C17
	Liver and bile ducts, gallbladder	21	C22-C24, C26.9
	Pancreas	34	C25
	Nasal cavity, middle ear, accessory sinuses, larynx, trachea	26	C30-C33
	Bone and articular cartilage of limb	1	C40-C41
	Mesothelial and soft tissue	20	C45-C49
	Vulva or vagina	5	C51-C52
	Cervix uteri or corpus uteri	14	C53-C54
	Ovarium	5	C56-C57
	Penis or testis	5	C60,C62-C63
	Eye, brain, and other parts of central nervous system	8	C69-C72
	Thyroid gland	3	C73
	Ill-defined, secondary and unspecified sites	22	C76-C80

Appendix 2. Tumor histopathology according to ICD-O-3 classification

Histopathology according to ICD-O-3 code	Number
800 Neoplasms, not further specified	60
801-804 Epithelial neoplasms, not further specified	83
805-808 Squamous cell neoplasms	117
809-811 Basal cell neoplasms	1
812-813 Papillomas and transitional cell carcinomas	61
814-838 Adenomas and adenocarcinomas	568
843 Mucoepidermoid neoplasms	1
844-849 Cystic, mucinous and serous neoplasms	42
850-854 Ductal and lobular neoplasms	79
855 Acinar cell neoplasms	9
856-857 Complex epithelial neoplasms	1
872-879 Nevi and melanomas	55
880 Tumors of soft tissue and sarcomas, not further specified	2
881-883 Fibrous neoplasms	1
885-888 Lipomatous neoplasms	1
889-892 Myxomatous neoplasms	2
893-899 Complex mixed and stromal neoplasms	4
905 Mesothelial neoplasms	11
906-909 Germ cell neoplasms	3
912-916 Tumors of blood vessels	1
918-924 Neoplasms of bone and cartilage	1
938-948 Gliomas	4
949-952 Neuroepithelial neoplasms	1
959-972 Hodgkin lymphomas and non-Hodgkin lymphomas	33
973 Plasma cell tumors	15
976 Immunoproliferative diseases	1
980-994 Leukemias	32
998 Myelodysplastic syndrome	1

Appendix 3. Definitions of endpoint recurrent cardiovascular disease

Cardiovascular disease	Definition
Myocardial infarction	Myocardial infarction, fatal or non-fatal
Stroke	Cerebral infarction, fatal or non-fatal Intracranial hemorrhage, fatal or non-fatal Fatal stroke, undefined hemorrhage/infarction
Vascular mortality	Fatal stroke Fatal myocardial infarction Terminal heart failure Fatal rupture aneurysm abdominal aorta Sudden death Other vascular death
Cardiovascular disease	Any of the above



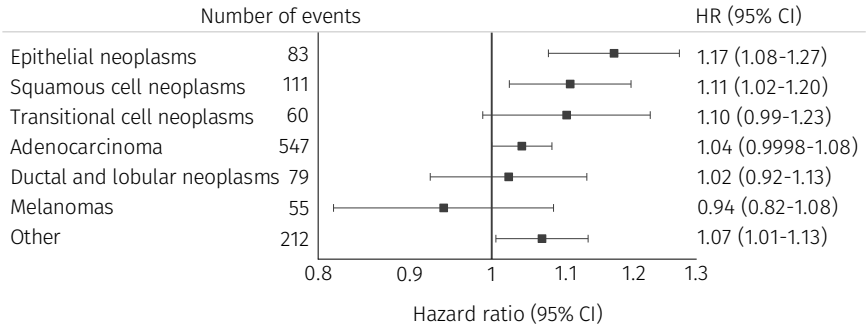
Appendix 4. Relation between CRP and recurrent cardiovascular disease risk

Cardiovascular disease is defined as the occurrence of myocardial infarction, stroke, or vascular mortality. Models are adjusted for age, sex, smoking status, number of pack-years, body mass index, LDL cholesterol, diabetes mellitus, systolic blood pressure, and kidney function. Continuous analyses represent hazard ratios per 1 mg/L higher CRP concentration.

Appendix 5. Relation between continuous CRP (1 mg/L higher) and risk of all lung cancers and for different histopathological diagnoses

Lung cancer **Histopathological diagnosis**	**HR (95% CI)**
All lung cancers	
Number of events	226
Model 1	**1.20 (1.15-1.26)**
Model 2	**1.16 (1.10-1.22)**
Small cell lung cancer (SCLC)	
Number of events	33
Model 1	**1.25 (1.11-1.42)**
Model 2	**1.23 (1.08-1.40)**
Non-small cell lung cancer (NSCLC) (including adeno-, squamous cell, and large cell carcinoma)	
Number of events	164
Model 1	**1.19 (1.12-1.26)**
Model 2	**1.18 (1.11-1.25)**
Adenocarcinoma	
Number of events	85
Model 1	**1.19 (1.10-1.29)**
Model 2	**1.19 (1.09-1.29)**
Squamous cell carcinoma	
Number of events	47
Model 1	**1.20 (1.08-1.33)**
Model 2	**1.19 (1.07-1.33)**
Large cell carcinoma	
Number of events	29
Model 1	**1.18 (1.03-1.36)**
Model 2	1.15 (0.99-1.33)

Model 1= Adjusted for age, sex; Model 2= Adjusted for age, sex, number of pack-years, body mass index, LDL cholesterol, diabetes mellitus, systolic blood pressure, and kidney function. No adjustment for smoking status due to low number of lung cancer in non-smokers. HR=Hazard ratio; CI=confidence interval.
The relatively high percentage of large cell undifferentiated NSCLC is related to the fact that the SMART cohort started in 1996 and histopathological subdivision of NSCLC was not routinely determined yet.



Appendix 6. Relation between continuous CRP and incident cancer according to histopathology, irrespective of anatomical location of origin

Hazard ratios are adjusted for age, sex, smoking status, number of pack-years, body mass index, LDL cholesterol, diabetes mellitus, systolic blood pressure, and kidney function. Category other includes basal cell neoplasms, mucoepidermoid neoplasms, cystic, mucinous, and serous neoplasms, acinar cell neoplasms, fibrous neoplasms, lipomatous neoplasms, myxomatous neoplasms, complex mixed and stromal neoplasms, mesothelial neoplasms, germ cell neoplasms, tumors of blood vessels, bone and cartilage neoplasms, gliomas, neuroepithelial neoplasms, Hodgkin lymphomas and non-Hodgkin lymphomas, plasma cell neoplasms, leukemias, and myelodysplastic syndrome (Appendix 2).

Appendix 7. Relation between continuous CRP (1 mg/L higher) and cancer risk, for all cancers and according to anatomical location, with separate adjustment for BMI and smoking

Endpoint of interest	HR (95% CI)
Lung (N=226)	
Model 1	**1.20 (1.15-1.26)**
Model 1b (smoking adjusted)	**1.14 (1.09-1.20)**
Model 1c (body mass index adjusted)	**1.22 (1.16-1.28)**
Model 2	**1.16 (1.10-1.22)**
Colon/rectum (N=177)	
Model 1	**1.06 (1.00-1.13)**
Model 1b (smoking adjusted)	1.05 (0.99-1.12)
Model 1c (body mass index adjusted)	1.06 (0.995-1.13)
Model 2	1.05 (0.98-1.12)
Urinary tract (N=107)	
Model 1	**1.10 (1.02-1.19)**
Model 1b (smoking adjusted)	1.08 (0.997-1.17)
Model 1c (body mass index adjusted)	**1.10 (1.01-1.19)**
Model 2	1.08 (0.995-1.17)
Lymphoid/hematopoietic (N=82)	
Model 1	**1.11 (1.01-1.21)**
Model 1b (smoking adjusted)	**1.11 (1.02-1.21)**
Model 1c (body mass index adjusted)	**1.11 (1.02-1.21)**
Model 2	**1.12 (1.02-1.22)**
Breast (N=69/1828)	
Model 1	1.04 (0.94-1.15)
Model 1b (smoking adjusted)	1.05 (0.95-1.16)
Model 1c (body mass index adjusted)	1.05 (0.95-1.16)
Model 2	1.06 (0.96-1.17)
Prostate (N=188/5350)	
Model 1	0.95 (0.89-1.02)
Model 1b (smoking adjusted)	0.95 (0.89-1.03)
Model 1c (body mass index adjusted)	0.96 (0.89-1.03)
Model 2	0.96 (0.89-1.03)
All cancers (N=1072)	
Model 1	**1.08 (1.05-1.11)**
Model 1b (smoking adjusted)	**1.06 (1.03-1.09)**
Model 1c (body mass index adjusted)	**1.08 (1.06-1.11)**
Model 2	**1.07 (1.04-1.09)**

Model 1= Adjusted for age, sex; Model 1b= Adjusted for age, sex, smoking status, and pack-years. Model 1c= Adjusted for age, sex, and body mass index. Model 2= Adjusted for age, sex, smoking status, pack-years, body mass index , LDL cholesterol, diabetes mellitus, systolic blood pressure, and kidney function. HR=Hazard ratio; CI=confidence interval; CVD=cardiovascular disease

Appendix 8. Relation between continuous CRP (1 mg/L higher) and cancer risk, stratified for smoking status

	Never smokers	**Former smokers**
	N= 1587	N=3416
	HR (95% CI)	HR (95% CI)
Lung		
Number of events	9	93
Model 1	1.18 (0.91-1.53)	**1.28 (1.19-1.37)**
Model 2	1.16 (0.88-1.53)	**1.26 (1.16-1.36)**
Colon/rectum		
Number of events	33	94
Model 1	**1.21 (1.06-1.38)**	1.07 (0.97-1.16)
Model 2	**1.19 (1.04-1.37)**	1.05 (0.95-1.15)
Urinary tract		
Number of events	13	55
Model 1	0.87 (0.62-1.22)	1.09 (0.97-1.22)
Model 2	0.90 (0.64-1.26)	1.04 (0.92-1.18)
Lymphoid/hematopoietic		
Number of events	19	42
Model 1	0.99 (0.79-1.25)	**1.19 (1.06-1.33)**
Model 2	0.94 (0.73-1.21)	**1.16 (1.03-1.32)**
Breast		
Number of events/females	23/563	28/648
Model 1	1.15 (0.97-1.35)	1.03 (0.89-1.20)
Model 2	1.16 (0.98-1.38)	1.02 (0.87-1.20)
Prostate		
Number of events/males	34/1024	112/2768
Model 1	0.85 (0.69-1.07)	0.96 (0.88-1.06)
Model 2	0.90 (0.72-1.12)	0.96 (0.87-1.06)
All cancers		
Number of events	182	539
Model 1	1.05 (0.98-1.12)	**1.09 (1.05-1.13)**
Model 2	1.05 (0.98-1.13)	**1.07 (1.03-1.11)**
CVD and/or cancer		
Number of events	360	1080
Model 1	**1.07 (1.02-1.12)**	**1.09 (1.07-1.12)**
Model 2	**1.05 (1.00-1.11)**	**1.06 (1.03-1.09)**

Model 1= Adjusted for age, sex; Model 2= Adjusted for age, sex, number of pack-years (for former and current smokers), body mass index, LDL cholesterol, diabetes mellitus, systolic blood pressure, and kidney function.

CRP=C-reactive protein; HR=Hazard ratio; CI=Confidence interval; CVD=cardiovascular disease.

Current smokers N=2175 HR (95% CI)	**P-value for interaction** Former vs never smokers	**P value for interaction** Current vs never smokers
124 1.06 (0.99-1.14) **1.08 (1.01-1.16)**	0.51	0.56
50 0.97 (0.85-1.09) 0.95 (0.83-1.08)	0.07	**0.01**
39 1.11 (0.98-1.25) 1.12 (0.99-1.26)	0.28	0.21
21 1.07 (0.90-1.27) 1.09 (0.91-1.29)	0.21	0.60
18/617 0.97 (0.80-1.18) 0.99 (0.81-1.22)	0.40	0.22
42/1558 0.98 (0.86-1.12) 0.96 (0.83-1.10)	0.44	0.29
351 **1.05 (1.01-1.10)** **1.05 (1.01-1.10)**	0.59	0.99
767 **1.08 (1.05-1.11)** **1.08 (1.05-1.11)**	0.62	0.47

Appendix 9. Relation between continuous CRP (1 mg/L higher) and cancer risk after exclusion of diagnosis within 1, 2, and 5 year(s) after inclusion in the cohort

	Exclusion cancer diagnoses <1 year N=7076 HR (95% CI)
Lung	
Number of events	213
Model 1	**1.19 (1.14-1.25)**
Model 2	**1.15 (1.09-1.21)**
Colon/rectum	
Number of events	159
Model 1	1.07 (1.00-1.14)
Model 2	1.05 (0.98-1.12)
Urinary tract	
Number of events	83
Model 1	**1.09 (1.00-1.19)**
Model 2	1.10 (0.998-1.20)
Lymphoid/hematopoietic	
Number of events	74
Model 1	1.08 (0.98-1.19)
Model 2	1.08 (0.98-1.20)
Breast	
Number of events/females	60/1800
Model 1	1.06 (0.96-1.17)
Model 2	1.08 (0.98-1.20)
Prostate	
Number of events/males	171/5276
Model 1	0.96 (0.89-1.03)
Model 2	0.96 (0.89-1.04)
All cancers	
Number of events	970
Model 1	**1.08 (1.05-1.11)**
Model 2	**1.06 (1.03-1.09)**
CVD and/or cancer (MI, stroke, vascular mortality, or cancer)	
Number of events	2078
Model 1	**1.10 (1.08-1.12)**
Model 2	**1.07 (1.05-1.09)**

Model 1= Adjusted for age, sex; Model 2= Adjusted for age, sex, smoking status, number of pack-years, body mass index , LDL cholesterol, diabetes mellitus, systolic blood pressure, and kidney function. CRP=C-reactive protein; HR=Hazard ratio; CI=Confidence interval.

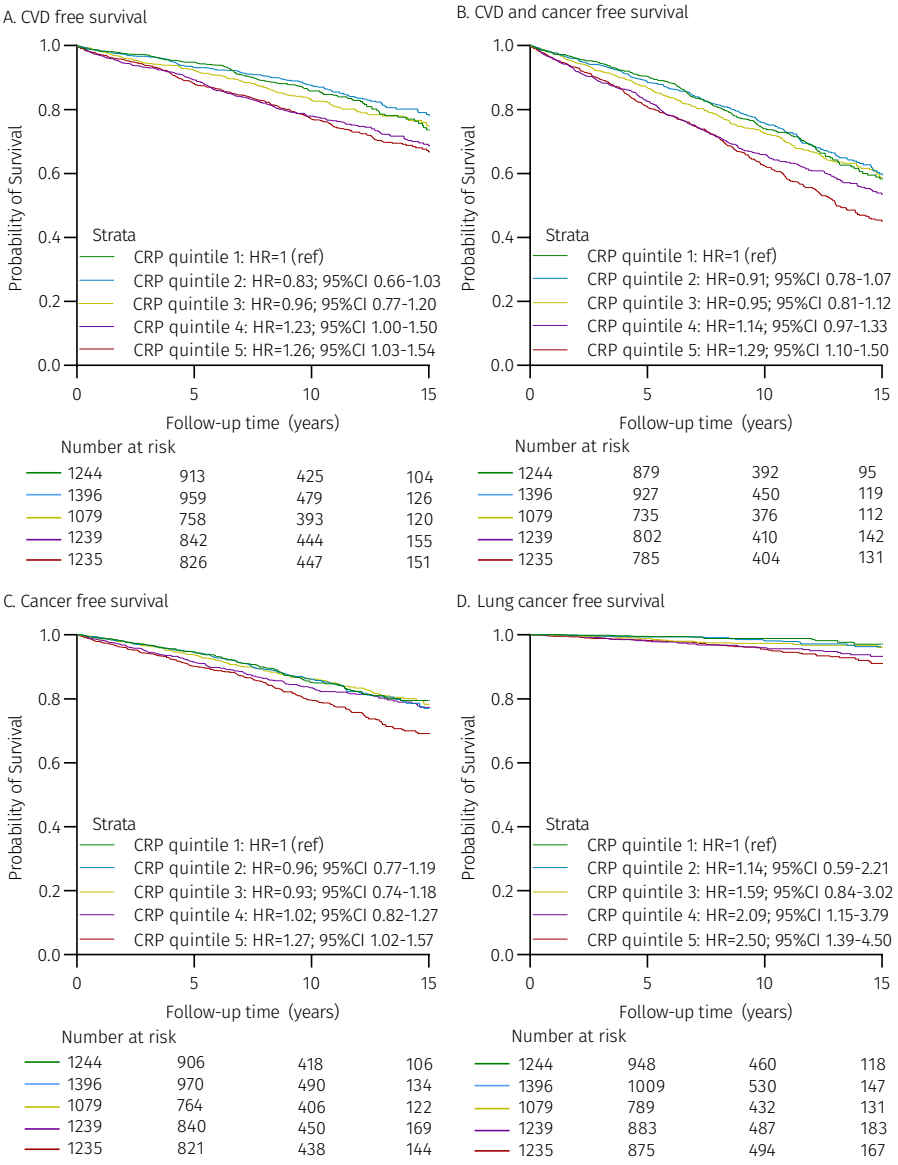
Exclusion cancer diagnoses <2 year N=6985 HR (95% CI)	**Exclusion cancer diagnoses <5 year** N=6701 HR (95% CI)
191 **1.20 (1.14-1.26)** **1.16 (1.09-1.23)**	124 **1.19 (1.12-1.28)** **1.15 (1.07-1.23)**
144 1.05 (0.98-1.13) 1.04 (0.96-1.12)	106 1.04 (0.95-1.13) 1.01 (0.92-1.11)
78 **1.10 (1.00-1.20)** 1.10 (0.997-1.21)	50 1.05 (0.93-1.18) 1.03 (0.91-1.18)
64 1.06 (0.95-1.18) 1.08 (0.97-1.20)	43 1.04 (0.91-1.19) 1.05 (0.91-1.21)
58/1780 1.08 (0.97-1.19) 1.10 (0.99-1.22)	44/1712 1.07 (0.95-1.20) 1.09 (0.97-1.23)
149/5205 0.97 (0.90-1.05) 0.98 (0.90-1.06)	92/4989 0.91 (0.82-1.02) 0.93 (0.83-1.04)
879 **1.07 (1.05-1.11)** **1.06 (1.03-1.09)**	595 **1.06 (1.02-1.10)** **1.04 (1.00-1.08)**
1987 **1.10 (1.08-1.12)** **1.07 (1.05-1.09)**	1703 **1.10 (1.08-1.12)** **1.07 (1.05-1.09)**

Appendix 10. Relation between continuous CRP (1 mg/L higher) and cancer risk stratified for cardiovascular disease at baseline

	Coronary artery disease (CAD) N= 3931 HR (95% CI)	**Cerebrovascular disease (CeVD)** N=1904 HR (95% CI)
Lung		
Number of events	89	49
Model 1	**1.30 (1.20-1.40)**	**1.16 (1.04-1.30)**
Model 2	**1.28 (1.18-1.38)**	**1.16 (1.03-1.31)**
Colon/rectum		
Number of events	98	46
Model 1	1.08 (0.99-1.19)	1.10 (0.98-1.24)
Model 2	1.04 (0.94-1.15)	1.09 (0.96-1.23)
Urinary tract		
Number of events	53	23
Model 1	1.08 (0.95-1.22)	1.10 (0.93-1.30)
Model 2	1.10 (0.96-1.25)	1.05 (0.87-1.26)
Lymphoid/ hematopoietic		
Number of events	42	15
Model 1	1.07 (0.93-1.23)	0.95 (0.75-1.22)
Model 2	1.10 (0.95-1.27)	0.97 (0.75-1.25)
Breast		
Number of events/females	31/728	21/720
Model 1	1.08 (0.93-1.26)	1.05 (0.88-1.25)
Model 2	1.07 (0.90-1.26)	1.05 (0.88-1.27)
Prostate		
Number of events/males	110/3203	47/1184
Model 1	0.95 (0.86-1.06)	1.01 (0.89-1.15)
Model 2	0.97 (0.87-1.08)	1.01 (0.88-1.17)
All cancers		
Number of events	540	261
Model 1	**1.10 (1.05-1.14)**	**1.06 (1.01-1.12)**
Model 2	**1.08 (1.04-1.13)**	**1.06 (1.00-1.12)**
CVD and/or cancer		
Number of events	1053	546
Model 1	**1.11 (1.08-1.14)**	**1.08 (1.05-1.12)**
Model 2	**1.08 (1.05-1.11)**	**1.06 (1.02-1.10)**

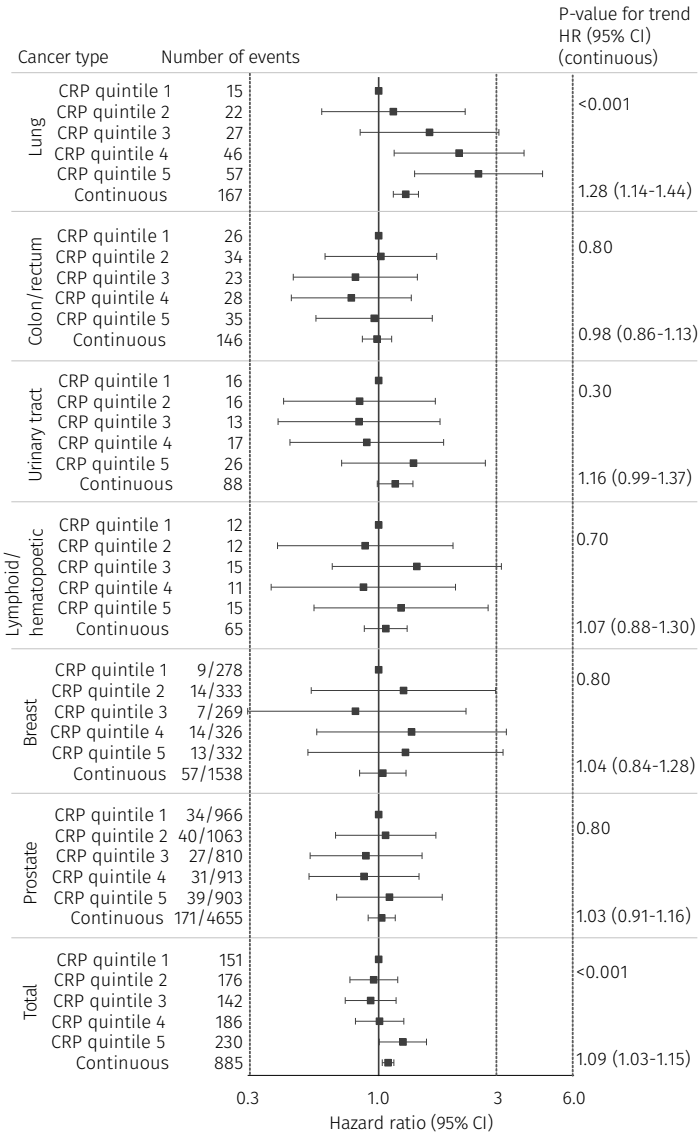
Model 1= Adjusted for age, sex; Model 2= Adjusted for age, sex, number of pack-years, smoking status, body mass index, LDL cholesterol, diabetes mellitus, systolic blood pressure, and kidney function. CRP=C-reactive protein; HR=Hazard ratio; CI=confidence interval; CVD=cardiovascular disease.

Peripheral vascular disease (PAD) N=1343 HR (95% CI)	**P-value for interaction** CeVD vs CAD	**P-value for interaction** PAD vs CAD
88 1.06 (0.98-1.15) 1.04 (0.95-1.13)	0.13	0.56
33 1.03 (0.90-1.18) 1.04 (0.91-1.19)	0.97	0.20
31 1.07 (0.93-1.22) 1.06 (0.93-1.22)	0.98	0.30
25 **1.17 (1.02-1.35)** **1.16 (1.00-1.34)**	0.21	0.17
17/380 0.94 (0.77-1.14) 0.96 (0.79-1.18)	0.45	0.75
31/963 0.91 (0.78-1.07) 0.89 (0.76-1.05)	0.65	0.21
271 1.05 (1.00-1.10) 1.04 (0.99-1.09)	0.39	0.99
591 **1.07 (1.03-1.10)** **1.06 (1.03-1.09)**	0.97	0.17



Appendix 11. Survival curves in CRP quintiles for recurrent cardiovascular disease, combined endpoint, total cancer, and lung cancer (only patients with CRP concentration ≤5 mg/L)

Hazard ratios are adjusted for age, sex, body mass index, smoking status, pack-years of smoking, LDL cholesterol, diabetes mellitus, systolic blood pressure, and kidney function. Quintile 1: CRP 0.49 (range 0.10-0.64); Quintile 2: CRP 0.90 (range 0.64-1.20); Quintile 3: CRP 1.50 (range 1.20-1.85); Quintile 4: CRP 2.35 (range 1.85-2.98); Quintile 5: CRP 3.80 (range 2.98-5.00).



Appendix 12. Relation between CRP and incident cancer, according to anatomical location of origin (only patients with CRP concentration ≤5 mg/L)

Hazard ratios are adjusted for age, sex, smoking status, number of pack-years, body mass index, LDL cholesterol, diabetes mellitus, systolic blood pressure, and kidney function. Analyses for breast and prostate cancer were performed in subgroups of women and men respectively. Number of events per number of women or men in CRP quintiles are given. Continuous analyses represent hazard ratios per 1 mg/L higher CRP concentration. Quintile 1: CRP 0.49 (range 0.10-0.64); Quintile 2: CRP 0.90 (range 0.64-1.20); Quintile 3: CRP 1.50 (range 1.20-1.85); Quintile 4: CRP 2.35 (range 1.85-2.98); Quintile 5: CRP 3.80 (range 2.98-5.00).

Chapter 3

Relation between healthy lifestyle changes and decrease in systemic inflammation in patients with stable cardiovascular disease

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Abstract

Background and aims

Pharmacological lowering of inflammation has proven effective in reducing recurrent cardiovascular event rates. Aim of the current study is to evaluate lifestyle changes (smoking cessation, weight loss, physical activity level increase, alcohol moderation, and a summary lifestyle improvement score) in relation to change in plasma CRP concentration in patients with established cardiovascular disease.

Methods

In total, 1794 patients from the UCC-SMART cohort with stable cardiovascular disease and CRP levels ≤10 mg/L who returned for a follow-up study visit after median 9.9 years (IQR 5.4-10.8) were included. The relation between changes in smoking status, weight, physical activity, alcohol consumption, a summary lifestyle improvement score and change in plasma CRP concentration was evaluated with linear regression analyses.

Results

Smoking cessation was related to a 0.40 mg/L decline in CRP concentration (β-coefficient -0.40; 95%CI -0.73,-0.07). Weight loss (per 1SD=6.4 kg) and increase in physical activity (per 1 SD=48 MET hours per week) were related to a decrease in CRP concentration (β-coefficients -0.25; 95%CI -0.33,-0.16 and -0.09; 95%CI -0.17,-0.01 per SD). Change in alcohol consumption was not related to CRP difference. Every point higher in the summary lifestyle improvement score was related to a decrease in CRP concentration of 0.17 mg/L (β-coefficient -0.17; 95%CI -0.26,-0.07).

Conclusions

Smoking cessation, increase in physical activity, and weight loss are related to a decrease in CRP concentration in patients with stable cardiovascular disease. Patients with the highest summary lifestyle improvement score have the most decrease in CRP concentration. These results may indicate that healthy lifestyle changes contribute to lowering systemic inflammation, potentially leading to a lower cardiovascular risk in patients with established cardiovascular disease.

Introduction

Systemic low-grade inflammation plays a role in the development of atherothrombotic disease by initiating plaque formation, as well as stimulating plaque progression and transformation to vulnerable plaques that are more prone to erosion or rupture.[1] Epidemiological evidence further supports the role of low-grade inflammation in the development of lung cancer.[2-4] Pharmacological lowering of systemic inflammation, at least with an interleukin (IL)-1β antagonist, has recently been shown to reduce incidence rates of both cardiovascular events and lung cancer.[2,5]

C-reactive protein (CRP), an acute phase protein, is a part of the IL-1β, IL-6 inflammatory pathway,[6] and plasma CRP concentrations ≤ 10mg/L reflect systemic low-grade inflammation.[7] Several medical conditions, as well as lifestyle factors including smoking,[8] abdominal obesity,[9] physical activity,[10] and alcohol intake[11] influence systemic inflammation. Mechanisms include promotion of local pulmonary inflammation due to cigarette smoke by recruitment of natural killer cells and neutrophils from the microcirculation to the lungs,[12] leading to a systemic inflammatory response by secretion of pro-inflammatory mediators.[13] Adipose tissue production of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α), and IL-6 is increased as the visceral adipose tissue compartment expands.[14] Regular physical activity reduces systemic low-grade inflammation through three potential mechanisms including reduction of visceral adipose tissue, increased production of anti-inflammatory cytokines from contracting skeletal muscles, and reduced production of inflammatory cytokines by monocytes.[15] Chronic excessive alcohol use leads to increased production of pro-inflammatory cytokines due to alcoholic liver injury,[16] whereas light to moderate alcohol use compared to no alcohol is thought to reduce inflammation through ethanol-induced inhibition of pro-inflammatory cytokine and chemokine production, such as IL-6 and TNF, by circulating monocytes.[17,18]

Despite these associations, the effect of lifestyle improvements on reducing low-grade inflammation in patients with cardiovascular disease remains controversial. Although weight loss and physical activity have been shown to reduce CRP levels,[19-21] conflicting results are reported for effects of smoking cessation, diet, and alcohol consumption.[22-27]

The aim of the current study is to examine the association between lifestyle behaviors and systemic low-grade inflammation at baseline, as well as the relation between lifestyle changes (including smoking cessation, weight loss, physical activity level increase, alcohol moderation, and a summary lifestyle improvement score) and change in systemic low-grade inflammation, measured by CRP plasma concentrations, in a cohort of patients with established cardiovascular disease.

Patients and methods

Study population

Participants originated from the Utrecht Cardiovascular Cohort-Second Manifestations of ARTerial disease (UCC-SMART) cohort, an ongoing prospective cohort study that started in 1996. The UCC-SMART cohort includes 18 to 79 year-old patients referred to the University Medical Center (UMC) in Utrecht, the Netherlands. Study design and rationale have been described in detail previously.[28] From 2006 onwards, patients with at least 4 years of follow-up were invited for reassessment of baseline measurements (UCC-SMART-2 cohort). Yearly, approximately 350 consecutive patients of the original UCC-SMART-cohort were invited by mail, achieving a recruitment efficacy of 58% (Flowchart Appendix 1). Baseline characteristics of patients with a second visit compared to patients with a baseline visit only are shown in Appendix 2. The study complies with the Declaration of Helsinki, was approved by the University Medical Center's Ethics Committee and all patients provided written informed consent. For the current study, patients with established cardiovascular disease at baseline who returned for second measurements, and with CRP levels ≤ 10 mg/L at both visits were included (N=1794). Established cardiovascular disease was defined as cerebrovascular disease (transient ischemic attack, cerebral infarction, amaurosis fugax, retinal infarction, history of carotid surgery), coronary artery disease (angina pectoris, myocardial infarction, coronary revascularization), peripheral artery disease (symptomatic and documented obstruction of distal arteries, revascularization of the leg, amputation), or an aneurysm of the abdominal aorta (distal aortic anteroposterior diameter ≥3 cm, history of AAA surgery). Participants with CRP levels >10 mg/L were excluded (N= 217), as CRP levels >10 mg/L are commonly associated with an acute inflammatory response.[7] Time between visit and vascular event was at least two months (both baseline and follow-up measurement). Advice on lifestyle improvements was given according to general clinical practice, lifestyle interventions were not part of this observational cohort study.

Measurements at baseline and follow-up visit

The same data was acquired at baseline and follow-up visit following a standardized protocol. Information on smoking status (never, former, or current, and number of pack-years) and alcohol consumption (no alcohol, <1, 1-10, 11-20, 21-30, or >30 units per week) was obtained by a questionnaire. Weight was measured on traditional scales. A previously validated questionnaire suitable for ranking subjects[29] was used for measuring physical activity, with one additional question on the intensity of sports activity. Number of hours per week reported by patients for sports, walking, cycling, and gardening, was multiplied by a specific metabolic equivalent of task (MET) derived from the Compendium of Physical activity[30], resulting in a number of MET hours per week per activity. The total amount of physical activity was the sum of the MET hours per week of all activities. Work-related

physical activity (categories; sedentary occupation, standing occupation, manual labor, or heavy manual labor) and retirement status were additionally recorded. Information on dietary habits was not available.

Lifestyle changes and summary lifestyle improvement score

Achievement of lifestyle goals regarding smoking, weight, physical activity, and alcohol consumption according to cardiovascular disease prevention guidelines [31] was assessed at baseline and follow-up. Change in continuous lifestyle variables (weight, physical activity, and number of pack-years), as well as CRP, was determined by the difference between follow-up and first measurement. Changes in categorical variables were defined as smoking cessation (compared to continuing smoking), smoking start (compared to continued non-smoking), alcohol use change from heavy to moderate or no alcohol use (compared to continued heavy users), and alcohol use change from no alcohol to moderate (compared to continued none use). For the creation of a summary lifestyle improvement score, summing up the changes in the four lifestyle components, each lifestyle factor was graded; -1 for deterioration (e.g. started smoking or gained weight (>1SD)), 0 for no change (e.g. remained former smoker, similar alcohol use, weight and physical activity change within 1SD), and 1 for improvement (e.g. quit smoking or lost weight (>1SD)). The sum of the grades of the four lifestyle characteristics formed the summary lifestyle improvement score with a minimum of -4 and a maximum of 4, and was calculated for each individual patient.

Registration of events during follow-up

From the first visit onwards, patients received biannual questionnaires obtaining information on incident cardiovascular disease, bleeding events, diabetes mellitus, and end stage renal disease. Upon an affirmative answer, additional information was gathered through hospital or general practitioner's data. An endpoint committee of three physicians independently judged all clinical events, and conflicting decisions were discussed. Detailed information on definitions and number of endpoints is described in Appendix 3.

Data analyses

Missing data for smoking status (<0.3%), alcohol use (<1%), weight (<0.5%), CRP (<1.6%), use of lipid lowering and platelet inhibitory medication (<0.3%), and physical activity (<16%) were singly imputed by bootstrapping and predictive mean matching, based on multiple regression using both baseline and follow-up visit measurements as well as outcome data (aregImpute function in R, Hmisc package). With regard to the high percentage of missing physical activity data, a sensitivity analysis was performed with only complete cases regarding physical activity.

For descriptive statistics, a baseline table, histogram for the distribution of difference in CRP concentration and cross tables of CRP differences per lifestyle characteristic were created. Cross-sectional analyses at baseline were performed first for all lifestyle factors by linear regression analyses with CRP concentration at baseline as the dependent variable and lifestyle factors (smoking status, alcohol use, body mass index (BMI), and physical activity at baseline) as independent variables. To investigate the relation between lifestyle changes and change in CRP concentration, linear regression analyses were performed. Difference in CRP was taken as the dependent variable, and each change in lifestyle as independent variable. Continuous independent variables (weight change and physical activity change) were assessed per SD increase. For the categorical independent variables, continuous smokers, continuous heavy alcohol users, and continuous non alcohol users were taken as the reference category. Baseline CRP was added to the models, as the magnitude of the difference in CRP level might depend on baseline concentration. To adjust for potential confounding, age and sex, and additionally change in use of lipid lowering (including change in statin use) or antiplatelet medication, smoking status change, weight change, physical activity change, and alcohol use change (if not determinant of interest) were added to the models. Exploratory models were evaluated with addition of educational level, retirement between visits, change in work-related physical activity, estimated glomerular filtration rate (eGFR) change, systolic blood pressure change, diabetes at baseline, diabetes acquired during follow-up, and low density lipoprotein cholesterol (LDL-c) change were assessed, as well as additional adjustment for the use of hormone replacement therapy at baseline in women, or other anti-inflammatory medication (including non-steroidal anti-inflammatory drugs (NSAIDs), COX2 inhibitors, corticosteroids, and immunosuppressive medication).

The relation between multiple lifestyle changes and change in CRP concentration was evaluated by plotting mean difference (standard error of the mean (SEM)) of CRP versus the summary lifestyle improvement score, for all patients and stratified for CRP concentration at baseline (the median CRP level at baseline of 1.5 mg/L was chosen as cut-off value). Furthermore, linear regression was performed with the summary lifestyle improvement score as a continuous independent variable and CRP difference as dependent variable, adjusted for age, sex, CRP at baseline, and change in use of lipid lowering or antiplatelet medication.

Additional analyses and assumptions of linear regression

Potential effect modification by time since smoking status was tested by adding an interaction term to the model. Estimated marginal means of CRP concentration were calculated for never smokers and patients who quit smoking during follow-up, adjusted for age, sex, change in other lifestyle factors, and lipid lowering and antiplatelet medication. To evaluate potential effects of incident cancer (N=105) or cardiovascular disease (N=126) during the follow-up period on the relation between lifestyle changes and change in CRP, a sensitivity analysis was performed by excluding these patients.

Assumptions of linear regression; linearity between independent variable and outcome, normality of residuals, and homogeneity of variance were assessed visually and no violations were observed.

Results

Baseline characteristics

In total, 1794 patients with clinically manifest cardiovascular disease and CRP levels ≤ 10 mg/L were included. Mostly males were included (79%), due to the specific study population of patients with established CVD. Median time between the first and follow-up study visit was 9.9 years (interquartile range (IQR) 5.4-10.8 years). Patient characteristics for the first and follow-up visit are shown in Table 1. Median CRP concentration was 1.5 mg/L (IQR 0.8-3.1) at baseline and 1.4 mg/L (IQR 0.7-2.7) at follow-up, and CRP levels were fairly stable with a mean difference of -0.18 mg/L (SEM 0.05) between the first and follow-up study visits (Appendix 4).

Change in lifestyle between baseline and follow-up

At baseline and follow-up only 5% and 4% of the patients had achieved all four lifestyle goals for smoking, physical activity, BMI, and alcohol intake, even though slight improvements were observed for smoking and BMI (Appendix 5). The majority of the patients did not change their lifestyle habits during follow-up, regarding smoking, physical activity, weight, and alcohol use (Appendix 6). At baseline, 520 (29%) patients were current smokers. During follow-up, 261 patients quit smoking whereas 51 patients started smoking. Most patients had a stable weight comparing baseline and follow-up (N = 1327 (74%)), and the majority of patients had a stable level of physical activity (N=1364 (76%)). Although most patients did not change their alcohol intake (N=1493 (83%)), 203 patients moderated alcohol use from more than 10 units to fewer than 10 per week (Table 2).

Relation between lifestyle changes and change in CRP concentration

Cross-sectional analyses at baseline showed that smoking status, and BMI were associated with CRP concentration at baseline. Alcohol consumption and physical activity were not associated with baseline CRP concentration (Appendix 7).

Table 1. Patient characteristics at the first and follow-up study visit

Population, n=1794	**First visit**	**Follow-up visit***
Male, n (%)	1409 (79%)	1409 (79%)
Age (years)**	57 ± 9	66 ± 9
Cerebrovascular disease, n (%)	447 (25%)	485 (27%)
Coronary heart disease, n (%)	1181 (66%)	1242 (69%)
Peripheral vascular disease, n (%)	274 (15%)	323 (18%)
Diabetes Mellitus, n (%)	220 (12%)	370 (21%)
Metabolic syndrome, n (%)	848 (47%)	953 (53%)
Current smoking, n (%)	520 (29%)	310 (17%)
Number of pack-years**	15 (3 - 30)	19 (4 - 34)
Alcohol use (> 10 units per week), n (%)	595 (33%)	490 (27%)
Physical exercise (MET hours/week)**	43 (25 - 71)	44 (25 - 72)
Medication		
Lipid lowering medication, n (%)	1204 (67%)	1485 (83%)
Blood pressure lowering medication, n (%)	1310 (73%)	1385 (77%)
Anti-platelet therapy, n(%)	1385 (77%)	1446 (81%)
Anti-coagulants, n (%)	137 (8%)	192 (11%)
Other anti-inflammatory medication, n (%)***	51 (3%)	117 (7%)
Physical examination		
Body Mass Index (kg/m^2)**	27 ± 4	27 ± 4
Waist circumference (cm)**	95 ± 11	98 ± 12
Systolic blood pressure (mmHg)**	139 ± 20	140 ± 17
Diastolic blood pressure (mmHg)**	82 ± 11	80 ± 10
Laboratory measurements		
Hs-CRP (mg/L)**	1.5 (0.8 - 3.1)	1.4 (0.7 - 2.7)
Triglycerides (mmol/L)**	1.4 (1.0 - 2.0)	1.2 (0.9 - 1.8)
HDL cholesterol (mmol/L)**	1.2 (1.0 - 1.4)	1.2 (1.0 - 1.5)
LDL cholesterol (mmol/L)**	2.8 (2.2 - 3.5)	2.4 (2.0 – 3.0)
eGFR (CKD-EPI, mL/min/1.73m^2)**	79 ± 15	75 ± 17

* Median time between visits 9.9 years (IQR 5.4-10.8 years)

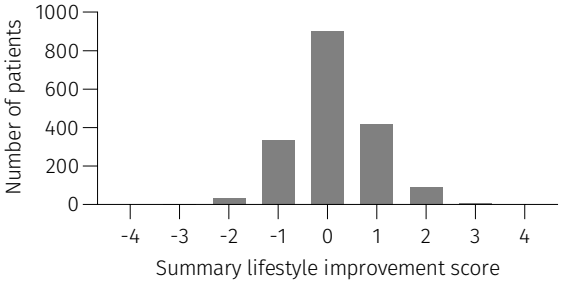
** Data are mean ±SD or median (interquartile range)

*** Other anti-inflammatory medication: non-steroidal anti-inflammatory drugs (NSAIDs), COX-2 inhibitors, colchicine, corticosteroids, and immunosuppressive medication (including methotrexate).

Table 2. Change in lifestyle factors between baseline and follow-up visit, separate and combined in the summary lifestyle improvement score

	Score		
	-1	**0**	**1**
Smoking status change*	51 (3%)	1479 (82%)	264 (15%)
Weight change**	291 (16%)	1327 (74%)	176 (10%)
Physical activity level change***	212 (12%)	1364 (76%)	218 (12%)
Alcohol use change****	98 (6%)	1493 (83%)	203 (11%)

Number of patients per summary lifestyle improvement score



* Smoking status change: started smoking (-1), no change (remained non-smoker or current smoker) (0), or quit smoking (1)

** Weight change: >1SD (=6.4kg) higher (-1), within 1SD (0), >1SD lower (1).

*** Physical activity change: >1SD (=48 METh/w) lower (-1), within 1SD (0), >1SD higher (1)

**** Alcohol use change: From ≤ 10 to > 10 units per week (-1), similar (0), from >10 to ≤10 units per week (1).

Smoking cessation was related to a 0.40 mg/L decline in CRP concentration (β-coefficient -0.40; 95%CI -0.73,-0.07) (Figure 1). No effect modification was observed by time since smoking cessation (p-value for interaction 0.97). Estimated marginal means of CRP level for participants who quit smoking during follow-up and for never smokers were 2.24 mg/L (95%CI 1.95,2.53) and 1.87 mg/L (95%CI 1.59,2.15) respectively. With regard to physical activity, for every SD increase in MET hours per week, CRP was 0.09 mg/L lower (β-coefficient -0.09; 95%CI -0.17,-0.01) (Figure 1). Similar results were observed after excluding patients with missing data on physical activity at the follow-up visit (β-coefficient -0.13; 95%CI -0.22,-0.04). Weight loss was related to a decrease in CRP concentration; per SD weight loss the CRP concentration decreased with 0.25 mg/L (β-coefficient -0.25; 95%CI -0.33,-0.16) (Figure 1). Change from heavy to moderate alcohol use was not related to CRP concentration (β-coefficient -0.22; 95%CI -0.52,0.09), and no relation was observed between change from no alcohol to moderate alcohol use and change in CRP level (β-coefficient -0.08; 95%CI -0.54,0.38) (Figure 1). Additional adjustment for use of hormone replacement therapy at baseline in women, diabetes mellitus at baseline, educational level, retirement between visits, change in work-related physical activity, or change in eGFR, systolic blood pressure, presence of diabetes mellitus, LDL-c, and anti-inflammatory medication use did not change results.

Analyses repeated after exclusion of patients with incident cardiovascular disease (n= 126) or any type of cancer, except for non-melanoma skin cancer (n= 105) between the two visits showed similar results.

Relation between summary lifestyle improvement score and change in CRP concentration

Patients with the highest summary lifestyle improvement scores (≥2) (N=99 (6%)) on average had the most decline in CRP concentration or, after stratification by baseline CRP concentration, the most favorable trend in CRP level (Figure 2). A linear relation was observed between summary lifestyle improvement score and CRP difference, when adjusted for baseline CRP, age, sex, and change in lipid lowering and antiplatelet medication. Every point higher was related to a decrease in CRP concentration of 0.17 mg/L (β-coefficient -0.17; 95%CI -0.26,-0.07). As no relation was observed between alcohol consumption and CRP, an additional analysis was performed for the summary lifestyle improvement score without incorporation of alcohol use, showing similar results.

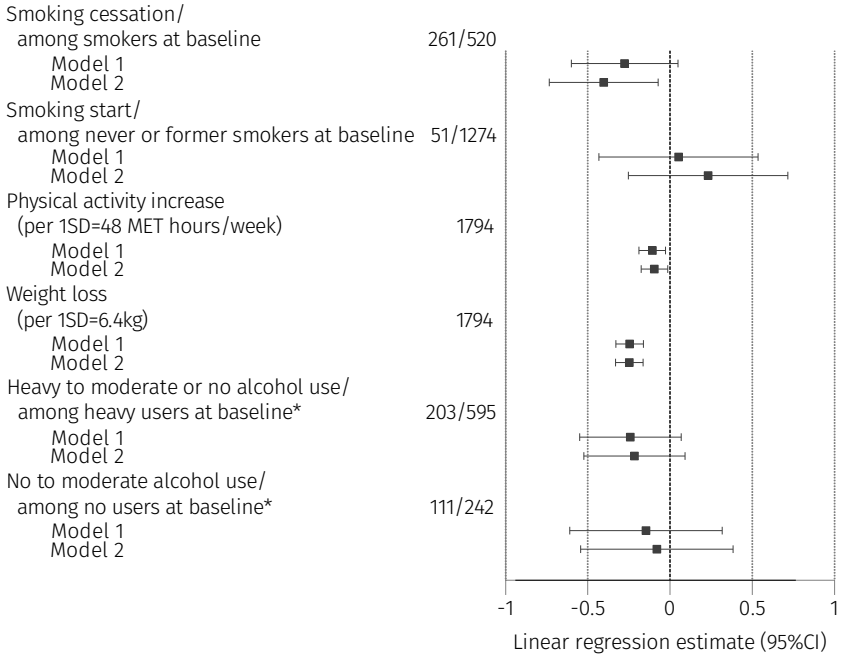


Figure 1. Relation between lifestyle changes and change in CRP concentration

Model 1: adjusted for age, sex, and CRP concentration at baseline

Model 2: additionally adjusted for difference in: smoking status, physical activity level, weight, alcohol use (if not determinant of interest), and change of lipid lowering and antiplatelet therapy.

* Heavy alcohol use: >10 units per week; Moderate alcohol use: >0-10 units per week.

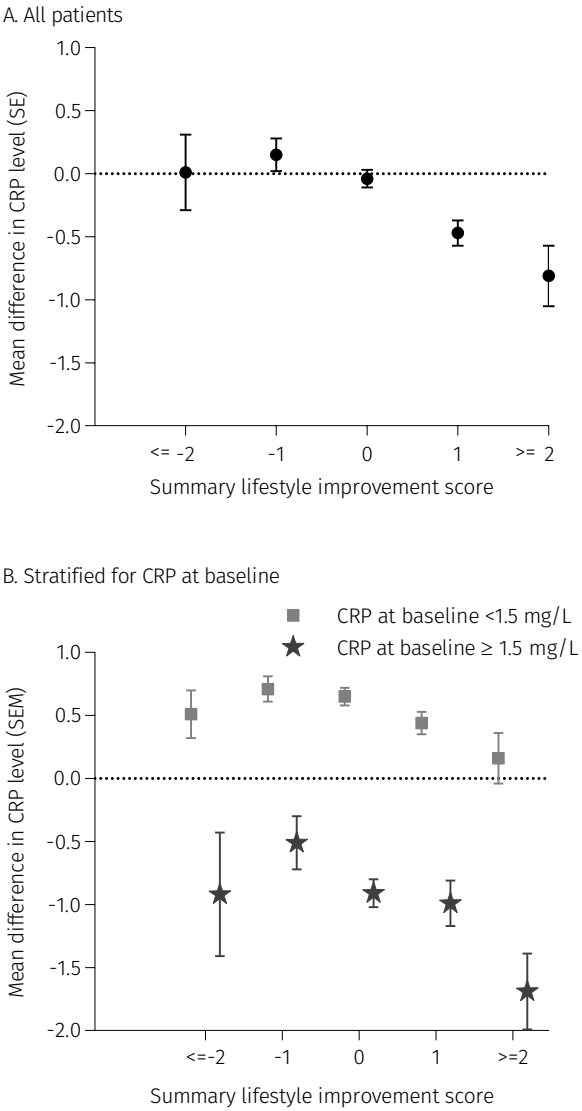


Figure 2. Relation between summary lifestyle improvement score and change in CRP concentration

Change in CRP level is difference between follow-up visit and baseline.

Discussion

In the present study it is shown that lifestyle factors smoking and body mass index are associated with systemic low-grade systemic inflammation at baseline and that smoking cessation, increase in physical activity, and weight loss are related to a decrease in CRP plasma concentration in patients with established cardiovascular disease. Alcohol use and change in alcohol use were not associated with CRP plasma concentration. Every point higher in the summary lifestyle improvement score, a combination of changes in lifestyle factors, was related to a further decrease in CRP concentration.

Results of the present study support the notion that lifestyle factors and lifestyle changes are related to low-grade systemic inflammation, potentially explaining part of the beneficial effects of lifestyle changes on reduction of cardiovascular risk. Results for weight loss and physical activity increase are in line with previous studies in population based cohorts and trial populations for lifestyle interventions.[19,20] Inconsistencies were observed for smoking cessation and alcohol consumption.[22-24] Smoking cessation was not related to change in CRP after one year in a longitudinal smoking cessation trial with 1504 participants[24], or to change in CRP after an average of 3.4 years (range 1.0-10 years) in 975 smokers at baseline.[22] However, smoking cessation was accompanied by an increase in waist circumference,[24] which may have counterbalanced CRP lowering effects of smoking cessation, and was not apparently taken into account in the analyses.[22,24] In the current study, smoking cessation was not related to CRP difference in crude analysis, only after adjustment for weight change, the relation became apparent. Moderate alcohol intake compared to no alcohol use was previously related to lower CRP concentrations in cross-sectional or trajectory analyses in population based studies[11,23] and in a subgroup of patients with a history of cardiovascular disease (N=1154).[32] In patients who consumed fewer than 7 drinks per week[32] or less than 20g of ethanol (corresponding to 0.5L beer) daily[11] CRP concentration was lowest. In the current study, moderate alcohol intake (>0-10 drinks per week) was not related to CRP concentration, implying that the upper limit of moderate alcohol use might be fewer than 10 drinks per week in order to have a beneficial effect on CRP concentration. Dietary information was not available in the present study. The relation between diet composition and CRP is uncertain; in a trial randomizing patients to a dietary regiment type, weight loss was the main driver of lowering CRP levels, irrespective of diet composition,[25] whereas a cross-sectional observational study found a relation between dietary glycemic load and CRP independent of BMI.[26]

The Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) showed that targeting inflammation by canakinumab, an IL-1β inhibitor, lowered CRP levels and reduced the risk of recurrent CVD.[5] Furthermore, the Colchicine Cardiovascular Outcomes Trial (COLCOT) showed that lowering inflammation with colchicine in patients after a recent myocardial infarction

reduced the risk of ischemic cardiovascular events.[33] CRP was lower in the colchicine group, although not statistically significant, and inflammation markers were only determined in a small and selected subgroup of patients.[33] No influence on CRP levels, nor incident cardiovascular disease by methotrexate was observed in the Cardiovascular Inflammation Reduction Trial (CIRT).[34] The combined results of these trials, illustrate the involvement of the IL-1β, IL-6, CRP pathway in pathophysiology of atherothrombosis and lead to the hypothesis that cardiovascular disease risk reduction could be dependent on the targeted inflammatory pathway.[6,35] Since smoking cessation, weight loss, and increased physical activity showed a beneficial effect on CRP concentration, mechanisms by which lifestyle interventions lead to a decreased risk of recurrent cardiovascular disease might include reduction of low-grade systemic inflammation. Similarly, low-grade inflammation is considered a stimulating factor in lung cancer development,[2-4] and smoking cessation might lead to a decreased risk of lung cancer compared to continuous smokers,[36] partially through a reduction of low-grade inflammation. The effect of other lifestyle factors on lung cancer risk in smokers, such as weight and physical activity, is unclear.[37,38]

The relation between lifestyle changes and decrease in CRP concentration in patients with cardiovascular disease is important for clinical practice, as a healthy lifestyle is an important part of secondary prevention.[39] A previous cross-sectional study suggested that 38% of patients with coronary artery disease and high inflammatory burden could achieve CRP levels lower than 2 mg/L after assumed lifestyle optimisation.[40] However, in the current study, reflecting real life, most patients did not manage to optimize lifestyle. Therefore, patients might benefit from further encouragement or assistance with improving lifestyle habits. Patients with a CRP concentration of ≥1.5 mg/L at baseline and the most lifestyle improvements (summary lifestyle improvement score of 2 or 3) had a mean difference in CRP concentration of -1.69 mg/L (SEM 0.30). In the CANTOS trial, including cardiovascular patients with a CRP concentration of ≥2 mg/L at baseline, canakinumab 150mg lowered median CRP levels by 2.3 mg/L after 48 months follow-up (median 4.3 mg/L at baseline and 2.0 mg/L at follow-up), compared to a decrease of 0.5 mg/L in the placebo group.[5] Although this is a short term pharmacological intervention, these results suggest that patients with established cardiovascular disease potentially benefit from specific anti-inflammatory therapy, on top of healthy lifestyle changes, to lower cardiovascular risk.

Strengths of the study include the large study population of patients with cardiovascular disease and the repeated measurement of lifestyle factors and CRP concentration. Potential limitations should be considered and include the reported lifestyle habits by questionnaires at two time points (baseline and follow-up), which might not be representative of the complete follow-up period. However, lifestyle habits and CRP concentration are measured simultaneously, and CRP concentration will therefore be representative for lifestyle habits of the preceding weeks. The long duration between baseline and follow-up visit potentially limits clinical importance of lifestyle goals achievement. Social desirability bias could have influenced participants' answers

concerning physical activity, smoking, and alcohol consumption, leading to an underestimation of the relation with CRP concentration. Furthermore, the selection of patients who returned for follow-up measurements could lead to selection bias. The questionnaire to quantify physical activity is previously validated,[29] but not specifically for change in physical activity level. Furthermore, validation showed that the questionnaire is suitable for ranking subjects rather than calculating absolute energy expenditure,[29] and might be less suited for determining individual achievement of guideline recommended physical activity goals. Absence of elaborate information on daily alcohol intake (rather than weekly), may have influenced the results for alcohol consumption and CRP, and the relatively small number of patients changing from no to moderate alcohol intake (N= 111) could have limited precision. Unmeasured confounders, including diet, hormone replacement therapy at follow-up for women, additional comorbidities, or medication compliance could have influenced the results of the study. However, by studying the relation between difference in lifestyle and difference in CRP concentration within participants, effects of unmeasured confounding are potentially limited. Given the observational study design, firm conclusions on causality should be made with caution as residual confounding cannot be ruled out.

In conclusion, smoking cessation, increase in physical activity, and weight loss are related to a decrease in CRP concentration in patients with stable cardiovascular disease. Patients with the highest summary lifestyle improvement score have the most decrease in CRP concentration. These results may indicate that healthy lifestyle changes contribute to lower systemic inflammation, potentially leading to a lower cardiovascular risk in patients with stable cardiovascular disease.

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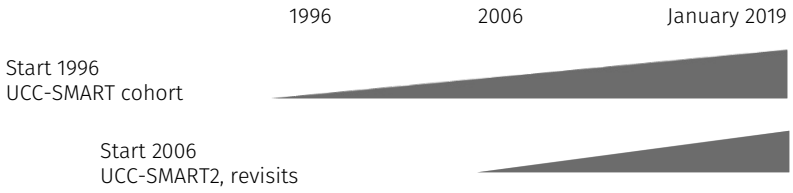
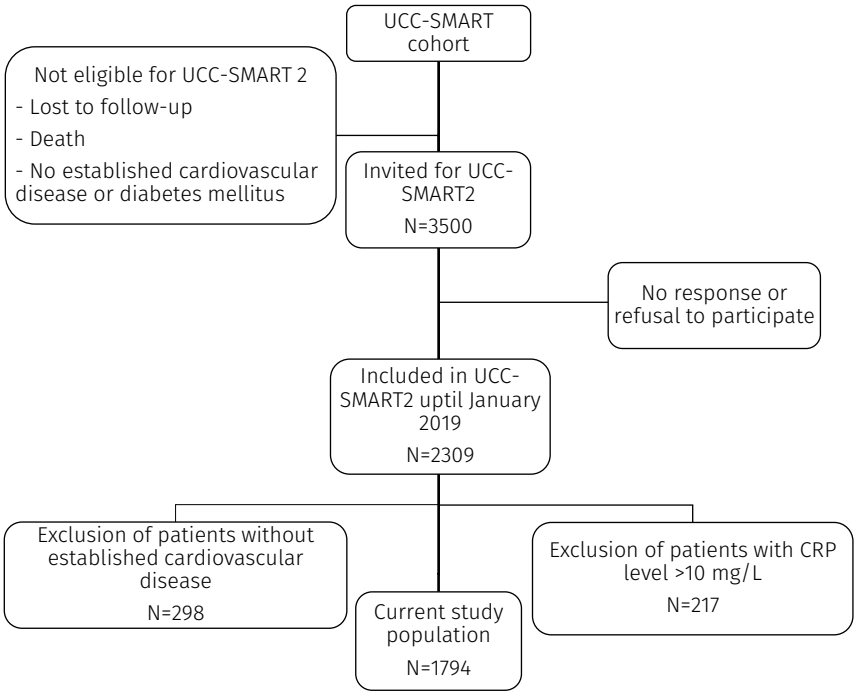
Appendices

A. Schematic representation of UCC-SMART2 cohort



B. Flowchart of inclusion



Appendix 1. Schematic representation and flowchart of UCC-SMART-2 cohort

Appendix 2. Baseline characteristics responders versus non-responders

	Responders N=1794	**Non-responders N=4323**
Male, n (%)	1409 (79%)	3137 (73%)
Age (years)*	57 ± 9	61 ± 11
Cerebrovascular disease, n (%)	447 (25%)	1362 (32%)
Coronary heart disease, n (%)	1181 (66%)	2546 (59%)
Peripheral vascular disease, n (%)	274 (15%)	872 (20%)
Diabetes Mellitus, n (%)	220 (12%)	839 (19%)
Metabolic syndrome, n (%)	848 (47%)	2330 (54%)
Current smoking, n (%)	520 (29%)	1413 (33%)
Number of pack-years*	15 (3 - 30)	16 (3 - 33)
Alcohol use (> 10 units per week), n (%)	595 (33%)	1212 (28%)
Physical exercise (MET hours/week)*	43 (25 - 71)	42 (23 - 69)
Medication		
Lipid lowering medication, n (%)	1204 (67%)	2891 (67%)
Blood pressure lowering medication, n (%)	1310 (73%)	3193 (74%)
Anti-platelet therapy, n(%)	1385 (77%)	3275 (76%)
Anti-coagulants, n (%)	137 (8%)	509 (12%)
Physical examination		
Body Mass Index (kg/m^2)*	27 ± 4	27 ± 4
Waist circumference (cm)*	95 ± 11	96 ± 12
Systolic blood pressure (mmHg)*	139 ± 20	141 ± 21
Diastolic blood pressure (mmHg)*	82 ± 11	81 ± 11
Laboratory measurements		
Hs-CRP (mg/L)*	1.5 (0.8 - 3.1)	2.0 (1.0 - 3.8)
Triglycerides (mmol/L)*	1.4 (1.0 - 2.0)	1.4 (1.0 - 2.0)
HDL cholesterol (mmol/L)*	1.2 (1.0 - 1.4)	1.2 (1.0 - 1.4)
LDL cholesterol (mmol/L)*	2.8 (2.2 - 3.5)	2.7 (2.1 - 3.5)
eGFR (CKD-EPI, mL/min/1.73m^2)*	79 ± 15	75 ± 18

Non-responders are patients with clinically manifest vascular disease at baseline and CRP level ≤10 mg/L, included before June 2012 (most recent baseline visit for patient included in SMART2 was in May 2012).
* Data are mean ±SD or median (interquartile range)

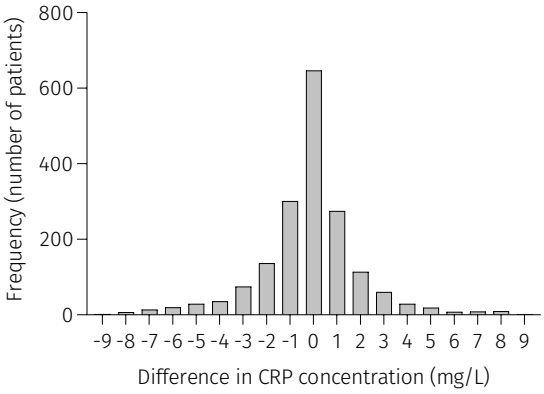
Appendix 3. Endpoint definitions and number during follow-up

	Definition [1]	**Number between baseline and FU visit**
Non-fatal myocardial infarction	At least two of the following: 1. Chest pain for at least 20 minutes, not disappearing after administration of nitrates; 2. ST-elevation >1 mm in two following leads or a left bundle branch block on the ECG; 3. CK elevation of at least two times the normal value of CK and a MB-fraction >5% of the total CK.	87
Non-fatal stroke	Relevant clinical features which have caused an increase in handicap of at least one grade on the modified Rankin scale,accompanied by fresh infarct or hemorrhage on a repeat CT scan	43
Vascular death	Sudden death (unexpected cardiac death occurring within 1 hour after onset of symptoms, or within 24 hours given convincing circumstantial evidence) or death from stroke, myocardial infarction, congestive heart failure, rupture of abdominal aortic aneurysm, or from other cause, i.e. sepsis following stent placement.	NA
Cardiovascular disease (combined endpoint)	Non-fatal myocardial infarction or stroke (vascular death not applicable)	126

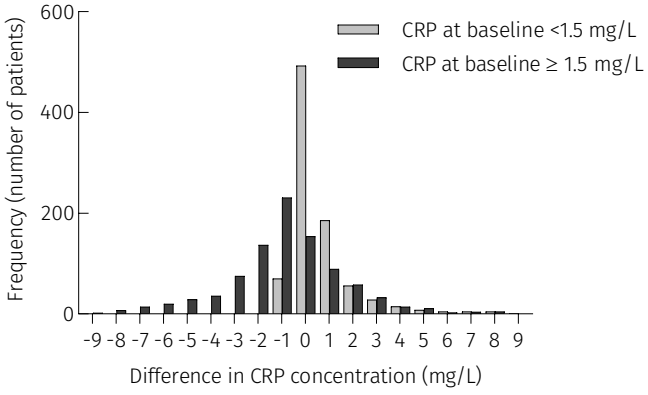
Reference

1. Simons PC, Algra A, van de Laak MF, Grobbee DE, van der Graaf Y. Second manifestations of ARTerial disease (SMART) study: rationale and design. *Eur J Epidemiol* 1999; 15(9): 773-81.

A. All patients



B. Stratified for CRP concentration at baseline



Appendix 4. Histogram of difference in CRP level between baseline and follow-up measurement

Appendix 5. Achievement of treatment goals according to cardiovascular disease prevention guidelines*, at baseline and follow-up

	Baseline	**Follow-up**
Former or never smoker**	1274 (71%)	1484 (83%)
Physical activity from sports ≥ 11.25 METh/week***	458 (26%)	430 (24%)
Body mass index ≤25 kg/m^2 ****	606 (34%)	519 (29%)
Alcohol intake <10 units per week (men) and <1 per week (women) *****	1028 (57%)	1144 (64%)
All four targets achieved	89 (5%)	70 (4%)

* Guidelines: "2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR)"[1]

** No exposure to tobacco in any form is recommended.

*** At least 150 minutes a week of moderate physical activity (30 minutes for 5 days/week) or at least 75 minutes a week of vigorous activity (15 minutes for 5 days/week) or a combination thereof is equivalent to 11.25 MET hours per week; one hour moderate-intensity activity corresponds with 4.5 MET, resulting in a recommended weekly MET of at least 11.25 (4.5 times 2.5 hours). Only physical activity from sports included.

**** BMI 20-25 kg/m^2 is recommended. Patients with a BMI between 18 and 20 kg/m^2 were also considered on target.

***** Alcohol intake was available as categorical variable with options 0 (no alcohol), 1 (<1 per week), 2 (1-10 per week), 3 (>10-20 per week), 4 (>20-30 per week), 5 (>30-40 per week), 6 (>40 per week). For women guidelines advise not more than one unit daily, and therefore <1 per week was regarded as according to guidelines. For men, guidelines advise not more than 2 alcoholic units daily, and ≤10 units was regarded as following guidelines.

Appendix 6. Number of patients and difference in CRP concentration per lifestyle factor at baseline and follow-up

A. Smoking status

		Follow-up		
		Never smoker	**Former smoker**	**Current smoker**
Baseline	**Never smoker**	N= 328	N= 10	N= 3
		ΔCRP 0.02 (±1.93)	ΔCRP -1.29 (±2.44)	ΔCRP 0.41 (±1.38)
	Former smoker	N= 0	N= 885	N= 48
		ΔCRP NA	ΔCRP -0.11 (±2.12)	ΔCRP -0.67 (±2.68)
	Current smoker	N= 0	N= 261	N= 259
		ΔCRP NA	ΔCRP -0.31 (±2.12)	ΔCRP -0.41 (±2.48)

B. Physical activity*

		Follow-up		
		≤ 30.4 MET h/w	**30.4-59.8 MET h/w**	**>59.8 MET h/w**
Baseline	**≤ 30.4 MET h/w**	N= 258	N= 215	N= 125
		ΔCRP -0.11 (±2.17)	ΔCRP -0.20 (±2.31)	ΔCRP -0.58 (±2.40)
	30.4-59.8 MET h/w	N= 178	N= 209	N= 211
		ΔCRP 0.05 (±2.27)	ΔCRP -0.16 (±2.04)	ΔCRP -0.31 (±2.19)
	>59.8 MET h/w	N= 139	N= 190	N= 269
		ΔCRP -0.19 (±2.06)	ΔCRP -0.06 (±1.91)	ΔCRP -0.13 (±2.15)

C. Body mass index (BMI)

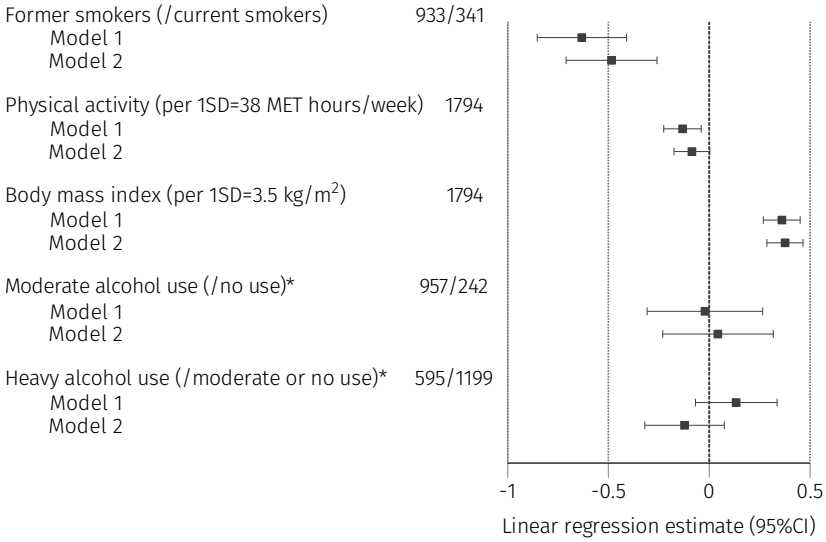
		Follow-up			
		BMI < 20 kg/cm²	**BMI 20-25 kg/cm²**	**BMI 25-30 kg/cm²**	**BMI > 30 kg/cm²**
Baseline	**BMI < 20 kg/cm²**	N= 12	N= 17	N= 0	N= 0
		ΔCRP -0.52 (±1.61)	ΔCRP 1.07 (±3.18)	ΔCRP NA	ΔCRP NA
	BMI 20-25 kg/cm²	N= 10	N= 359	N= 197	N= 8
		ΔCRP -0.32 (±1.91)	ΔCRP -0.11 (±1.83)	ΔCRP -0.11 (±2.07)	ΔCRP 0.60 (±2.35)
	BMI 25-30 kg/cm²	N= 1	N= 104	N= 644	N= 142
		ΔCRP -5 (±NA)	ΔCRP -0.47 (±2.12)	ΔCRP -0.21 (±2.23)	ΔCRP 0.11 (±2.16)
	BMI > 30 kg/cm²	N= 0	N= 1	N= 66	N= 233
		ΔCRP NA	ΔCRP -3 (±NA)	ΔCRP -0.78 (±2.40)	ΔCRP -0.20 (±2.38)

Appendix 6. Continued

D. Alcohol use

		Follow-up		
		No alcohol	**<1-10 units per week**	**>10 units per week**
Baseline	**No alcohol**	N= 130	N= 111	N= 1
		ΔCRP -0.25 (±2.08)	ΔCRP -0.24 (±2.06)	ΔCRP 3.35 (±NA)
	<1-10 units per week	N= 82	N= 778	N= 97
		ΔCRP -0.60 (±2.16)	ΔCRP -0.10 (±2.19)	ΔCRP -0.35 (±1.97)
	>10 units per week	N= 45	N= 158	N= 392
		ΔCRP -0.87 (±2.03)	ΔCRP -0.31 (±2.11)	ΔCRP -0.04 (±2.24)

* Physical activity categories based on 25th and 75th percentile. MET h/w = Metabolic equivalent of task hours/week. ΔCRP = Difference in C-reactive protein. Mean (standard deviation) is shown.



Appendix 7. Cross sectional analysis of the association between lifestyle factors and CRP concentration at baseline

Model 1: adjusted for age and sex at baseline. Model 2: additionally adjusted for baseline variables: smoking status, physical activity level, weight, alcohol use (if not determinant of interest), and lipid lowering and antiplatelet therapy. * Heavy alcohol use: >10 units per week; Moderate alcohol use: >0-10 units per week.

Part II

Cardiovascular calcification

Chapter 4

Multifocal cardiovascular calcification in patients with established cardiovascular disease; prevalence, risk factors, and relation with recurrent cardiovascular disease

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Abstract

Aims

The aim is to investigate (multifocal) cardiovascular calcification in patients with established cardiovascular disease (CVD), regarding prevalence, risk factors, and relation with recurrent CVD or vascular interventions. Coronary artery calcification (CAC), thoracic aortic calcification (TAC) (including ascending aorta, aortic arch, descending aorta), mitral annular calcification (MAC), and aortic valve calcification (AVC) are studied.

Methods

The study concerned 568 patients with established CVD enrolled in the ORACLE cohort. All patients underwent computed tomography. Prevalence of site-specific and multifocal calcification was determined. Ordinal regression analyses were performed to quantify associations of risk factors with cardiovascular calcification, and Cox regression analyses to determine the relation between calcium scores and recurrent CVD or vascular interventions.

Results

Calcification was multifocal in 76% (N=380) of patients with calcification. Age (per SD) was associated with calcification at all locations (lowest OR 2.17; 99%CI 1.54-3.11 for ascending aorta calcification). Diabetes mellitus and systolic blood pressure were associated with TAC, whereas male sex was a determinant of CAC. TAC and CAC were related to the combined endpoint CVD or vascular intervention (N=68). In a model with all calcium scores combined, only CAC was related to the combined outcome (HR 1.39; 95%CI 1.15-1.68).

Conclusion

Cardiovascular calcification is generally multifocal in patients with established CVD. Differences in associations between risk factors and calcification at various anatomical locations stress the divergence in pathophysiological pathways. CAC is most strongly related to recurrent CVD or vascular interventions independent of traditional risk factors, and independent of heart valve and thoracic aorta calcification.

Introduction

Cardiovascular calcification is a complicated and multifaceted process.[2] Risk factors and relation with incident cardiovascular disease have been studied in population based studies or apparently healthy people.[3-8] However, etiology of cardiovascular calcification is not yet completely understood and the relation between calcification and recurrent cardiovascular events in patients with established cardiovascular disease (CVD) specifically is unknown.

Pathophysiology of calcium deposition is an active process and varies between tissues. Calcification of the mitral valve occurs primarily in the mitral annulus (mitral annulus calcification (MAC)), the fibrous base of the mitral valve,[9] whereas aortic valve calcification (AVC) mainly affects the cusps.[10] In the vasculature, deposition of calcium in either the tunica media or tunica intima of the arterial wall are discrete forms of calcification; intimal calcification is generally related to atherosclerotic risk factors including hyperlipidemia and smoking,[2] whereas medial calcification is mainly influenced by diabetes mellitus and renal dysfunction.[11] Similar pathways exist in valvular and vascular calcification, including differentiation of resident cell population to osteoblast-like bone producing cells, and the loss of calcification inhibitors,[2,9,10,12] eventually leading to ectopic bone formation.[2,9,10]. However, the impact of risk factors on initiation and progression of cardiovascular calcification differs,[9-13] potentially leading to calcification in one location but not in another. Comparing associations of risk factors with calcification in multiple anatomical locations directly could provide insight in varying impact.

Regardless of pathophysiology, cardiovascular calcification; coronary artery calcification (CAC) as well as thoracic aorta calcification (TAC), is related to a higher risk of cardiovascular mortality in the general population[14] and incident cardiovascular disease in apparently healthy people,[6,15-17] independent of general cardiovascular risk factors.

The aim of the present study is to investigate (multifocal) cardiovascular calcification in patients with established vascular disease, with regard to (I) the prevalence of CAC, TAC (including ascending aorta, aortic arch, and descending aorta), MAC, and AVC, (II) the associations of predetermined cardiovascular risk factors with calcification of these anatomical locations in a direct comparison, and (III) the relation between cardiovascular calcification and recurrent cardiovascular disease and vascular interventions.

Methods

Study population

Patients originated from the ORACLE study (Clinicaltrials.gov Identifier NCT01932671), embedded in the UCC-SMART cohort. The UCC-SMART cohort is an ongoing prospective cohort study including 18-79 year-old patients referred to the University Medical Center in Utrecht (the Netherlands) with clinically manifest atherosclerotic vascular disease or marked risk factors. Study design and rationale have been described in detail previously.[18] Patients enrolled in the UCC-SMART cohort from August 2012, without contra-indications for contrast enhanced computed tomography, were invited to participate in the ORACLE study, consisting of non-contrast enhanced cardiac computed tomography (CT) and CT-angiography (CTA) visualizing the aortic arch to the circle of Willis. Contra-indications were reduced renal function (estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2), previous severe allergic reaction to contrast, previous exposure to radiation for scientific purposes or any other known contra-indication for CT-scanning. Information about the determinants and potential confounders was collected at baseline, following the UCC-SMART protocol, entailing thorough investigation of medical history, laboratory, physical and radiological examinations. Kidney function was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[19] The study is in accordance with the 1964 Helsinki declaration, was approved by the institutional review board of the Utrecht University Medical Center, and all patients gave written informed consent. For the current study, 568 patients with established cardiovascular disease enrolled in the ORACLE study were included.

CT-scan protocol and image analysis

Images were acquired using a 256-slice MDCT-scanner (iCT, Philips Healthcare, the Netherlands) on the same day as the baseline measurements. Non-contrast enhanced cardiac CT-scan, as well as coronary CT angiography images were acquired. Upon completion of the coronary CTA, a second acquisition with a new contrast injection was performed to visualize the vascular system from the aortic arch to the circle of Willis. Detailed information on the CT-scan protocol is summarized in Appendix 1. Calcification scoring was performed manually by an observer trained by an experienced radiologist and blinded for patient characteristics. Calcification of heart valves, coronary arteries, and ascending and descending aorta was scored on the non-contrast enhanced cardiac CT visualizing heart base to the pulmonary artery bifurcation. The aortic arch was scored on the contrast enhanced scan, as it was not included in the non-contrast cardiac scan. Due to the different scan settings for the contrast enhanced scan, calcium scores of aortic arch could not be added up to calcium scores of ascending and descending aorta and were therefore analyzed separately. Calcifications on valves and in the thoracic aorta were quantified using a “pseudo-mass” score, calculated

by multiplying the mean calcium HU value by the region of interest (ROI) volume for every lesion, and summing up the scores of all the lesions. CAC was quantified using the Agatston method.[20] Detailed information on image analysis is summarized in Appendix 1. Appendix 2 shows examples of calcification scoring for each location.

Incident cardiovascular disease or vascular interventions

Participants received biannual questionnaires during follow-up, gathering information on occurrence of recurrent CVD, bleeding events, incident diabetes, end stage renal disease, and hospitalizations for vascular interventions. Additional information was gained by collecting hospital or general practitioner's data. An endpoint committee of three physicians independently adjudicated all cardiovascular disease events and conflicting classifications were discussed. Experienced research nurses classified all vascular interventions. Cardiovascular disease was defined as non-fatal myocardial infarction, non-fatal stroke or vascular death. Cardiovascular interventions were percutaneous interventions or revascularization surgery, including carotid endarterectomy (CEA), percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), major amputations, and peripheral artery stenting, angioplasty or bypass. (Appendix 3 for outcome definitions).

Data preparation

Continuous risk factors were truncated to the 99th percentile to correct for outliers. Missing data was singly imputed for waist circumference (1.1%), LDL-c (0.2%), kidney function (0.2%), triglycerides (0.2%), hsCRP (1.2%), number of pack-years (0.2%), and HDL-c (0.2%) using bootstrapping and predictive mean matching based on multivariable regression with independent variable and outcome data. Calcium scores were categorized (Appendix 4). Categories for coronary artery calcification were based on clinical cut-off values: 0, 1-99, 100-399, and 400 or higher.[21] Since no clinical cut-off values were available for the other locations, calcium scores of ascending aorta, descending aorta, aortic arch, and aortic valve were divided into three categories: 0 if no calcification was present (calcium scores < 1 were considered no calcification), 1 and 2 for calcium scores ≥1 and lower or higher than the 50th percentile. Mitral annulus calcium scores were dichotomized (0 = no calcification and 1 = calcification), due to the low prevalence. Presence of multifocal calcification was summarized in the 'calcium sum score'. The sum score was calculated by adding up presence of calcification in the six locations, resulting in a minimum of 0 (no calcification in any of the locations), and a maximum of 6 (presence of calcification in all 6 structures) for every patient individually.

Data analyses

Prevalence and risk factors of cardiovascular calcification

Frequencies of the calcium sum score were calculated and plotted in histograms accordingly, for all patients combined, and after stratification by sex, age higher or lower than 60 years (mean age of the study population), and presence of diabetes mellitus. A cross table was created

showing prevalence of calcification in groups of patients with CAC, MAC, AVC, and TAC. Potential risk factors for calcification were predetermined and included age, sex, diabetes mellitus, number of pack-years of smoking, waist circumference, systolic blood pressure, pulse pressure, LDL-c, HDL-c, triglycerides, hsCRP, and kidney function. To explore the association of these risk factors with the presence and extent of cardiovascular calcification per location separately, regression analyses were performed. Due to the excess of zero scores (no calcification), ordinal regression by means of the proportional odds model was performed,[22] and logistic regression for MAC, with the ordered categories as outcome (Appendix 4). The proportional odds model results in a single odds ratio representing the association of a risk factor with the presence and extent of calcification per location. For continuous risk factors, odds ratios were given per one SD higher of that risk factor. To account for multiple testing, 99% confidence intervals (CI) were calculated around the estimates. Potential confounding was addressed by adjusting for age and sex, and additionally for systolic blood pressure, LDL-c, pack-years of smoking, kidney function, and diabetes mellitus, if not the determinant of interest. Models exploring the association between triglycerides and calcification were additionally adjusted for HDL-c. As more men entered the cohort with existing coronary heart disease (CHD), and more women with cerebrovascular disease, association of sex and calcification was assessed in strata of previous vascular disease; coronary heart disease (N=408) and cerebrovascular disease (N=165).

Relation between cardiovascular calcification and recurrent cardiovascular disease
To assess the relation between cardiovascular calcification at the different locations and incident CVD or the combined endpoint of CVD or vascular intervention, Cox proportional hazards regression analyses were performed. Calcium scores of the different locations, CAC, AVC, MAC, and TAC (ascending aorta, aortic arch, and descending aorta separately) were implemented as continuous determinants, as individual variables in separate models, and as individual variables in one model combined. Hazard ratios were given per one SD higher calcium score. Additionally, the relation between the calcium sum score and incident CVD was assessed. Traditional atherosclerotic risk factors; age, sex, systolic blood pressure, LDL-c, pack-years of smoking, kidney function, and diabetes mellitus were included in the models for the combined outcome. Due to the limited number of recurrent CVD specifically (N=15), only univariable analysis was performed for this outcome.

Model assumptions of the proportional odds, logistic regression, and Cox regression models were assessed (detailed description in Appendix 1) and no violations were observed. Data analyses were performed in R statistical software (version 3.5.1).

Results

Baseline characteristics

In total 568 patients, 126 women and 442 men, underwent CT imaging (Table 1). The majority of the patients were previously diagnosed with coronary heart disease (72%). Most of the patients were treated with lipid lowering medication (85%), blood pressure lowering agents (81%), and/or anti-platelet medication (88%).

Table 1. Baseline characteristics of the study population

Total number of patients	568
Men (%)	442 (78%)
Age (years)*	58 ± 10
Cerebrovascular disease, n (%)	165 (29%)
Coronary heart disease, n (%)	408 (72%)
Peripheral vascular disease, n (%)	29 (5%)
Diabetes Mellitus, n (%)	65 (11%)
Metabolic syndrome, n (%)	274 (48%)
Number of pack-years*	9 (0 - 24)
Physical examination and laboratory measurements	
Body Mass Index (kg/m²)*	27 ± 4
Waist circumference (cm)*	96 ± 12
Systolic blood pressure (mmHg)*	129 ± 15
Diastolic blood pressure (mmHg)*	78 ± 9
Pulse pressure (mmHg)*	51 ± 11
Common carotid intima-media thickness (cm)*	0.8 ± 0.2
Triglycerides (mmol/L)*	1.3 (1.0 - 1.8)
LDL-cholesterol (mmol/L)*	2.3 (1.9 - 2.9)
HDL-cholesterol (mmol/L)*	1.2 (1.0 - 1.4)
Hs-CRP (mg/L)*	1.4 (0.7 - 3.3)
eGFR (CKD-EPI, mL /min/1.73m²)*	89 ± 12
Medication	
Lipid lowering medication, n (%)	483 (85%)
Blood pressure lowering agents, n (%)	458 (81%)
Anti-platelet therapy, n (%)	500 (88%)
Anti-coagulants (vitamin K-antagonists), n (%)	37 (7%)

* Data are mean (± standard deviation) or median (interquartile range)

Prevalence of (multifocal) arterial and heart valve calcifications

55 (10%) patients had no calcifications in any of the anatomical locations, whereas 25 (4%) of the patients had calcium depositions in all anatomical locations (coronary arteries, mitral and aortic valve, and thoracic aorta simultaneously) (Appendix 4). CAC was most common, with a prevalence of 83%. Distributions of the calcium sum score are shown in Appendix 5. Stratification by sex resulted in similar distributions of the calcium sum score in men and women, whereas stratification by age or diabetes mellitus showed higher calcium sum scores in patients with higher age, and patients with diabetes mellitus (Appendix 5). Of the patients with calcification, in 76% (N=380) calcification was multifocal. Patients with MAC were most likely to have calcification in other locations simultaneously, whereas patients with CAC were least likely to have calcification in other locations (Table 2).

Table 2. Prevalence of calcification per anatomical location, in groups of patients with CAC, MAC, AVC, and TAC

Total N= 568	**Coronary artery calcification N= 474**	**Mitral annulus calcification N= 72**	**Aortic valve calcification N= 276**	**Thoracic aorta calcification N= 364**
Coronary artery calcification N= 474	474 (100%)	67 (93%)	260 (95%)	335 (92%)
Mitral annulus calcification N= 72	67 (14%)	72 (100%)	59 (21%)	66 (18%)
Aortic valve calcification N= 276	260 (56%)	59 (82%)	276 (100%)	231 (64%)
Thoracic aorta calcification N= 364	335 (71%)	66 (92%)	231 (84%)	364 (100%)
No calcification in other locations	96 (20%)	1 (1%)	6 (2%)	18 (5%)

Association between risk factors and calcification

For all of the investigated anatomical locations (coronary arteries, ascending and descending aorta, aortic arch, and heart valves), a higher age (per one SD) was associated with presence and greater extent of calcification (lowest OR 2.17; 99%CI 1.54-3.11 for ascending aorta calcification) (Figure 1). Except for age, no association was observed between any of the other risk factors and MAC or AVC. Number of pack-years was a determinant of CAC and TAC (OR 1.26; 99%CI 1.02-1.57 for CAC, OR 1.43; 99%CI 1.13-1.81 for ascending aorta calcification, OR 1.81; 99%CI 1.42-2.32 for aortic arch, and OR 1.59; 99%CI 1.25-2.04 for descending aorta calcification). Male sex was associated with CAC (OR 2.66; 99%CI 1.57-4.55). The association with male sex was no longer observed after stratification for type of CVD at baseline. Diabetes mellitus was strongly associated with aortic arch calcification (OR 2.21; 99%CI 1.08-4.58) and descending aorta calcification (OR 3.04; 99%CI

1.43-6.56). Associations with diabetes mellitus and calcification of coronary arteries and heart valves were slightly weaker and not statistically significant. Associations with systolic blood pressure were observed in descending aorta (OR 1.56; 99%CI 1.22-2.01) and aortic arch calcification (OR 1.34; 99%CI 1.05-1.70). No associations were observed with LDL-c, HDL-c, triglycerides, hsCRP, and kidney function (Appendix 6).

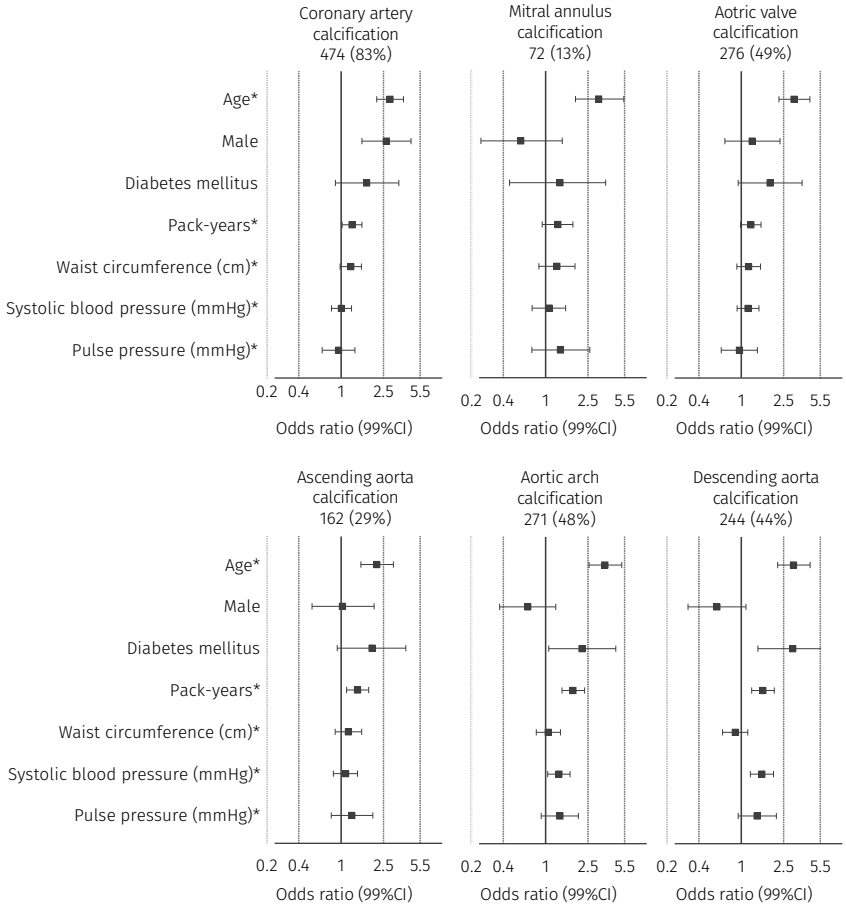

Figure 1. Associations of risk factors with presence and extent of calcification per anatomical location
Results from ordinal regression and logistic regression for mitral annulus calcification are shown. Models are adjusted for age, sex, pack-years of smoking, systolic blood pressure, LDL cholesterol, kidney function, and diabetes mellitus (if not the determinant of interest). * For continuous variables, odds ratios per one SD are given.

Relation between cardiovascular calcification and incident cardiovascular disease and interventions

During a median follow-up time of 2.74 years (IQR 1.55-3.96) 15 recurrent cardiovascular events were observed, of which 6 non-fatal strokes, 7 non-fatal myocardial infarctions, and 2 vascular deaths. Total number of events in the combined endpoint was 68 (5 non-fatal strokes, 6 non-fatal myocardial infarctions, 2 carotid artery interventions, 42 cardiac interventions, 11 peripheral artery interventions, and 2 vascular deaths).

TAC and CAC were related to incident CVD in univariable analysis (highest HR for CAC of 1.51; 95%CI 1.14-1.99) (Appendix 7). TAC and CAC were also related to the combined endpoint incident CVD or vascular intervention (Table 3). CAC showed the strongest relation with the combined endpoint (HR 1.35; 95%CI 1.15-1.58). Furthermore, in a model with calcium scores of all the different locations combined, the relation between CAC and the combined endpoint was the only to remain statistically significant (HR 1.39; 95%CI 1.15-1.68 respectively).

Table 3. Relation between cardiovascular calcification and combined endpoint of incident cardiovascular disease or vascular intervention (N=68)

	Calcium scores in separate models	**All calcium scores combined in one model**
	HR (95% CI)	HR (95% CI)
Coronary artery calcification	**1.35 (1.15-1.58)**	**1.39 (1.15-1.68)**
Mitral annulus calcification	1.09 (0.90-1.31)	0.79 (0.35-1.80)
Aortic valve calcification	0.91 (0.69-1.19)	1.01 (0.79-1.29)
Ascending aorta calcification	**1.19 (1.06-1.34)**	1.21 (0.96-1.53)
Aortic arch calcification	**1.16 (1.03-1.31)**	1.10 (0.70-1.73)
Descending aorta calcification	**1.15 (1.03-1.28)**	0.87 (0.56-1.37)
Calcium sum score	1.18 (0.84-1.67)	-

All models include age, sex, LDL cholesterol, pack-years of smoking, diabetes mellitus, kidney function, and systolic blood pressure. HR per 1SD higher calcium score are given. HR = hazard ratio. CI = confidence interval.

Discussion

Cardiovascular calcification of the coronary arteries, thoracic aorta and heart valves was common (90%) and generally multifocal in patients with established cardiovascular disease. Although cardiovascular calcification was generally multifocal, only a small percentage of patients (4%) showed calcium depositions in all of the locations simultaneously, and 10% had no calcification. Male sex was associated with CAC, whereas diabetes and systolic blood pressure were most strongly associated with TAC. Relation between calcium scores and incident CVD or vascular intervention was most pronounced for CAC.

Even though cardiovascular calcification is a systemic process, only 4% of the patients had calcifications in all studied anatomical locations, supporting hypotheses of divergence in pathophysiological processes.[13] For valvular calcification, no associations were observed with cardiovascular risk factors other than age, in contrast with arterial calcification. These results suggest that atherosclerotic processes, despite some contribution,[7-10] are less prominent in the initiation and progression of valvular calcification, and other pathophysiological pathways might be more influential. Growing evidence supports the role of increased mechanical stress as an important initiator of valvular calcification, by causing endothelial injury as well as accelerated degeneration of collagen and elastin fibers.[9,10] Vascular calcification can be characterized as intimal and medial calcification.[11] In the current study, systolic blood pressure and diabetes were most strongly associated with thoracic aorta calcification, in contrast with coronary artery calcification. Since systolic blood pressure and diabetes mellitus are both predominantly linked to media calcification,[11] these results suggest involvement of the tunica media layer in TAC. For blood pressure, the association is presumably two directional; hypertension might induce endothelial damage in the thoracic aorta, influencing atherosclerosis and intimal calcification,[23] but more importantly, medial calcification could cause vascular stiffness and a subsequent rise in blood pressure.[23] hyperglycemia in diabetes stimulates transformation of vascular smooth muscle cells to osteoblast-like cells via multiple pathways, including enhanced expression of osteoblast transcription factors.[11,24]

There is debate about whether calcium scores represent a reflection of total plaque burden and that calcification is simply a consequence ('scar tissue') of the atherosclerotic process, or that calcification is causally related to cardiovascular disease.[2,12] For intimal calcification, calcification of plaques might even serve as a stabilizer, preventing acute atherosclerotic events.[2] Regardless of which of these hypotheses is correct, the relation between calcification and incident cardiovascular disease is important for clinical practice. Similar to primary prevention,[15,25-27] in the current study coronary artery calcification and thoracic aorta calcification are related to incident CVD or vascular intervention independent

of atherosclerotic risk factors. The relation between coronary artery calcification and incident cardiovascular outcomes was most prominent, suggesting that CAC is the strongest predictor of recurrent CVD, and that if CAC is used in risk prediction, calcium scores of other locations might be redundant.

Patient with established cardiovascular disease are considered very high risk by guidelines,[1] however, the distribution of predicted 10-year risk of recurrent CVD is widespread[28] and a risk prediction models are available for these patients.[29,30] Although these patients will all require blood pressure and lipid management, accurate risk stratification could aid in clinical decision making regarding more aggressive risk factor treatment by expensive novel drugs, or lifestyle intervention programs.[28,30] Additionally, patients could be more accurately informed about their prognosis and risk of recurrence.[30] Future studies are needed to investigate the potential added prognostic value of CAC and extra-coronary calcification in the prediction of recurrent cardiovascular disease. Calcification can also be assessed by ultrasound,[31] and relation with cardiovascular events was observed in primary prevention patients.[32,33] It could be hypothesized that, even though ultrasound gives a semi-quantative score, ultrasound could also be appropriate to assess calcification for risk prediction in patients with established CVD.

This study had several strengths. First, calcification was measured in thoracic arteries, including the aortic arch, and in both heart valves in a specific study population of patients with established cardiovascular disease. Second, follow-up information was available in this prospective cohort study. Potential limitations of the study included the cross-sectional nature with regard to the risk factor analyses. Long-term effects of a determinant on the presence and extent of calcification could not be examined, and causal relations between the investigated risk factors and calcification could not be ascertained. Furthermore, the majority of the included patients with previous clinically manifest CVD used preventive medication, and baseline cholesterol levels and CRP might not reflect previous exposure, resulting in an underestimation of the association. Additionally, as patients were included based on sufficient kidney function due to contrast administration, no conclusions can be formulated regarding associations between low kidney function and cardiovascular calcification. Despite the large sample size of patients with a high prevalence of multifocal calcification, the prevalence and extent of calcification varied between the anatomical locations, leading to varying precision of the estimates. Especially the lower prevalence of aortic valve and mitral annular calcification might have led to more uncertainty compared to the other anatomical locations. Lastly, number of recurrent CVD events was relatively low (N=15). It could be hypothesized that the relation between calcium scores and CVD is different for incident stroke (N=5) and myocardial infarction (N=6) separately, however, too few events were observed for reliable analysis.

To conclude, cardiovascular calcification is generally multifocal in patients with established CVD. Differences in associations between risk factors and calcification at various anatomical locations stress the divergence in pathophysiological pathways. CAC is most strongly related to incident CVD or vascular intervention independent of traditional risk factors, and independent of heart valve and thoracic aorta calcification.

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Appendices

Appendix 1. Supplemental methods

CT-scan protocol

Non-contrast enhanced cardiac CT-scans were ECG gated with a standard tube voltage of 120 peak kilovoltage (kVp). To achieve most optimal imaging for the coronary CTA, patients whose heart rate exceeded 70 bpm were given beta-adrenergic blocking medication. A weight-dependent bolus of 70-90 ml iodine contrast was injected. Coronary CT angiography was performed using prospectively triggered protocols synchronized to the diastolic resting phase (78% of the RR-interval). In patients in whom the heart rate could not be lowered to 70 beats or less minute, or in cases of irregular heart rate retrospective gating was used.

Image analysis

For 7 patients, a non-contrast enhanced cardiac scan and for 7 patients the contrast enhanced scan of the aortic arch was absent, due to missing thin slice reconstruction, interfering artefacts, technical problems, or recent carotid or cardiac imaging. Patients with aortic valve replacement (n=9) were excluded from the analysis of the aortic valve, and patients with mitral valve replacement (n=6) were excluded from the analysis of the mitral annulus.

Thin slice reconstructions were created for scoring thoracic aorta and cardiac valve calcification with a slice thickness of 0.9 mm at 0.45 mm increment, and for coronary arteries a slice thickness of 3.0 mm at 3.0 mm increment was used according to Agatston guidelines.[1]

Calcification lesions were identified by the observer and the software (in-house developed software) automatically selected calcifications across consecutive slices. The automatic selection was inspected per slice and manually corrected if necessary, to prevent overestimation. Calcification in the aortic root was considered part of the aortic valve.[2] If a calcification lesion occupied multiple anatomical locations, it was scored according to its predominant location.

For the selection of calcification on the non-contrast enhanced scans a standard threshold of 130 Hounsfields Units (HU) was implemented. For the contrast enhanced scans, the threshold was set manually in each individual scan by making a selection in a contrast filled part in mid-arch, and adding 2 standard deviations (SD) to the mean HU of that selection.[3]

Data analysis

Prevalence and risk factors of cardiovascular calcification

Multiplicative interaction terms were added to the proportional odds models in order to test for potential effect modification by sex, showing no significant interactions (p-values >0.05). Additionally, to test for potential effect modification by medication use, multiplicative interaction terms with lipid lowering were added to models of LDL cholesterol, and with blood pressure lowering medication to models of systolic blood pressure, showing no significant interactions (p-values>0.05).

Model assumptions

The Brant test was used to assess the proportional odds assumption of the proportional odds model,[4] and no violations were observed (p-values >0.05). The linearity in the logit assumption for logistic regression was tested visually by adding the continuous independent variable of interest as a restricted cubic spline function to the model. No violations were observed.

For Cox regression analyses, proportional hazards assumption was assessed visually by plotting the scaled Schoenfeld residuals against time. Linearity was visually assessed by adding continuous calcium scores as restricted cubic spline functions to the model. No violations were observed.

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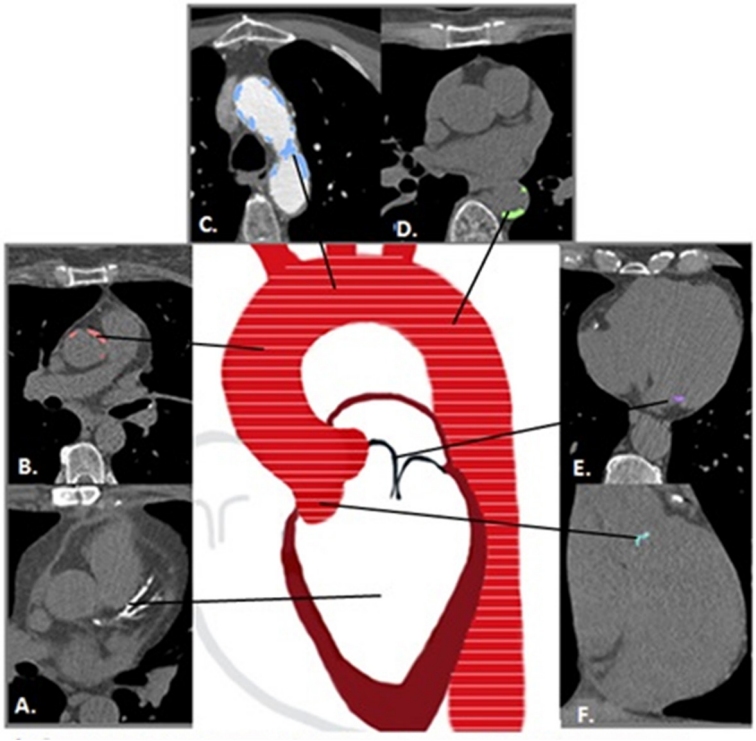

Appendix 2. Examples of calcification using the calcification scoring software

A. Coronary artery calcification; B. Ascending aorta calcification; C. Aortic arch calcification; D. Descending aorta calcification; E. Mitral annulus calcification; F. Aortic valve calcification.

Appendix 3. Outcome definitions in the UCC-SMART cohort

A. Definitions of incident cardiovascular disease [1,2]	
Non-fatal myocardial infarction	At least two of the following: 1. Chest pain for at least 20 minutes, not disappearing after administration of nitrates; 2. ST-elevation >1 mm in two following leads or a left bundle branch block on the ECG; 3. CK elevation of at least two times the normal value of CK and a MB-fraction >5% of the total CK.
Non-fatal stroke	Relevant clinical features which have caused an increase in handicap of at least one grade on the modified Rankin scale, accompanied by fresh infarct or hemorrhage on a repeat CT scan
Vascular death	Sudden death (unexpected cardiac death occurring within 1 hour after onset of symptoms, or within 24 hours given convincing circumstantial evidence) or death from stroke, myocardial infarction, congestive heart failure, rupture of abdominal aortic aneurysm, or from other cause, i.e. sepsis following stent placement.

B. Definition of incident vascular intervention*[1,2]	
Heart	Percutaneous coronary intervention or coronary artery bypass surgery
Carotid artery and intracranial arteries	Stent, angioplasty, (thrombo)endarterectomy, bypass surgery
Peripheral	Stent or graft (endovascular or open surgery), angioplasty, bypass surgery or (thrombo)endarterectomy. Major amputation (minor amputations of toe or forefoot are not included[3]) due to arterial ischemia. Other intervention due to ischemia (eg urokinase treatment, or emergency laparotomy due to intestinal ischemia)

* For the combined endpoint cardiovascular disease or intervention, patients who received a vascular intervention in response to a cardiovascular disease event, are classified according to the cardiovascular disease event

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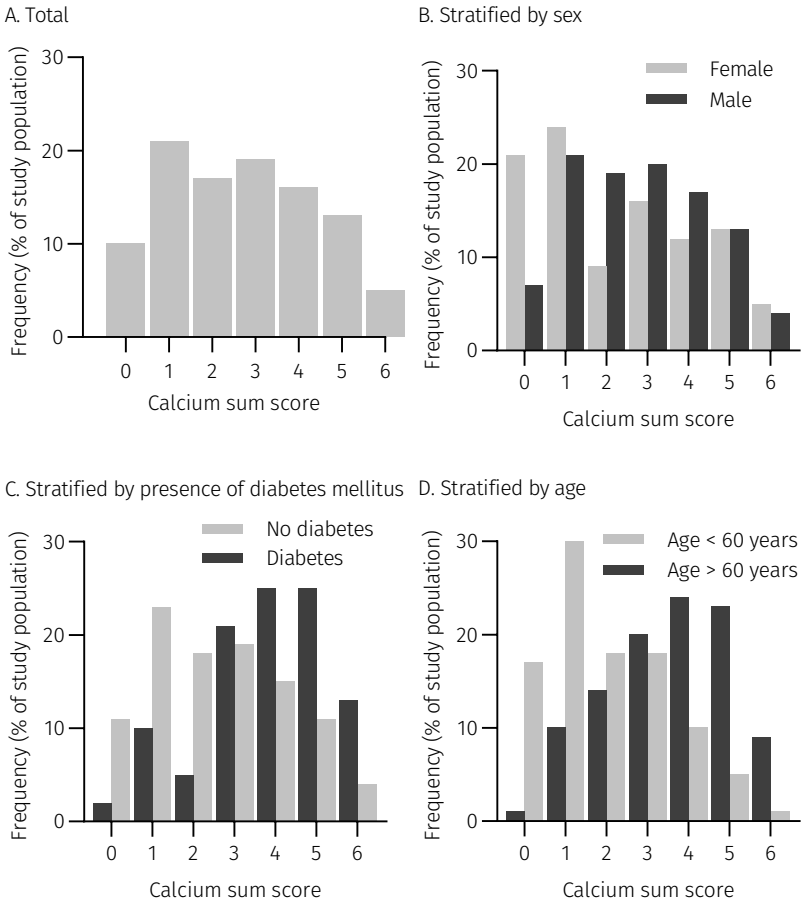
Appendix 4. Classification of categories and prevalence of calcification per location

	Category classification according to calcium score	**Number of patients (%) per category**	**Calcium score per category* (Median, IQR)**	**Number of lesions per category** (Median, IQR)**
No calcification in any of the locations	NA	55 (10%)	NA	NA
Calcification in all locations	NA	25 (4%)	NA	NA
Coronary arteries	0: No calcification	92 (16%)	NA	NA
	1: 1-99	125 (22%)	30 (9-67)	3 (2-6)
	2: 100-399	142 (25%)	210 (153-299)	13 (9-18)
	3: ≥ 400	207 (37%)	1008 (599-1634)	37 (26-50)
Ascending aorta	0: No calcification	399 (71%)	NA	NA
	1: 1-10	81 (14%)	3 (2-6)	1 (1-2)
	2: >10	81 (14%)	41 (18-79)	2 (2-4)
Aortic arch	0: No calcification	290 (52%)	NA	NA
	1: 1-91	136 (24%)	25 (6-43)	1 (1-3)
	2: >91	135 (24%)	251 (147-472)	4 (3-8)
Descending aorta	0: No calcification	317 (57%)	NA	NA
	1: 1-59	122 (22%)	11 (3-25)	2 (1-4)
	2: >59	122 (22%)	235 (113-551)	10 (6-15)
Aortic valve	0: No calcification	285 (51%)	NA	NA
	1: 1-17	138 (25%)	5 (3-10)	1 (1-2)
	2: >17	138 (25%)	50 (29-90)	4 (2-5)
Mitral annulus	0: No calcification	489 (87%)	NA	NA
	1: Calcification present	72 (13%)	18 (4-86)	1 (1-2)

* Agatston score for coronary arteries, (pseudo-)mass score for other anatomical locations.

** Number of lesions were determined in 3D. IQR = interquartile range; NA = not applicable



Appendix 5. Prevalence of multifocal calcification expressed by the calcium sum score

The sum score was calculated by adding up the presence of calcification of the six locations for every patient individually, resulting in a minimum of 0 (no calcification in any of the locations), and a maximum of 6 (calcification present in all 6 structures)

Appendix 6. Association of laboratory measurements with presence and extent of calcification per site

	Coronary artery calcification	**Mitral annulus calcification ****
LDL-c (mmol/L)*		
Model 1	**0.71 (0.58-0.87)**	1.07 (0.77-1.45)
Model 2	1.04 (0.81-1.32)	1.21 (0.78-1.86)
HDL-c (mmol/L)*		
Model 1	**0.79 (0.65-0.97)**	1.13 (0.82-1.54)
Model 2	0.92 (0.72-1.18)	0.93 (0.62-1.44)
Triglycerides (mmol/L)*		
Model 1	1.11 (0.91-1.36)	1.03 (0.73-1.39)
Model 2	1.12 (0.89-1.45)	1.12 (0.73-1.64)
CRP (mg/L)*		
Model 1	1.01 (0.83-1.23)	1.52 (0.86-1.52)
Model 2	1.04 (0.84-1.29)	1.15 (0.83-1.42)
eGFR (mL/min/1.73m^2)*		
Model 1	**0.65 (0.53-0.80)**	**0.59 (0.42-0.82)**
Model 2	1.07 (0.83-1.38)	0.90 (0.58-1.41)

LDL-c = Low density lipoprotein cholesterol; HDL-c = High density lipoprotein cholesterol, eGFR = estimated glomerular filtration rate, estimated by CKD-epi, CRP = C-reactive protein.
Model 1: Crude analysis. Model 2: Adjusted for age, sex, LDL cholesterol, number of pack-years, diabetes mellitus, kidney function, and systolic blood pressure (if not determinant of interest). Models for triglycerides were additionally adjusted for HDL cholesterol. Models for LDL-c were additionally adjusted for lipid lowering medication.
* For continuous variables, odds ratios per one SD increase are given. ** Logistic regression.

Appendix 7. Relation between cardiovascular calcification and recurrent cardiovascular disease (N=15)

	Calcium scores in separate models
	HR (95% CI)*
Coronary artery calcification	**1.51 (1.14-1.99)**
Mitral annulus calcification	1.14 (0.95-1.37)
Aortic valve calcification	1.22 (0.91-1.63)
Ascending aorta calcification	**1.31 (1.16-1.48)**
Aortic arch calcification	**1.26 (1.13-1.40)**
Descending aorta calcification	**1.24 (1.12-1.37)**
Calcium sum score	1.59 (0.91-2.77)

* Univariable analysis

	Aortic valve calcification	Ascending aorta calcification	Aortic arch calcification	Descending aorta calcification
	0.85 (0.69-1.06)	1.14 (0.90-1.42)	0.94 (0.76-1.15)	0.98 (0.80-1.21)
	1.13 (0.86-1.50)	**1.50 (1.11-2.04)**	1.01 (0.79-1.29)	1.16 (0.87-1.55)
	1.00 (0.81-1.23)	0.90 (0.70-1.14)	0.98 (0.80-1.21)	1.07 (0.87-1.33)
	1.09 (0.84-1.42)	0.78 (0.58-1.04)	0.92 (0.70-1.20)	1.00 (0.76-1.31)
	1.05 (0.85-1.28)	1.23 (0.98-1.54)	1.12 (0.92-1.37)	1.03 (0.83-1.27)
	1.11 (0.87-1.43)	1.14 (0.86-1.50)	1.05 (0.81-1.35)	0.95 (0.73-1.24)
	1.09 (0.88-1.34)	1.10 (0.88-1.37)	1.20 (0.98-1.48)	1.13 (0.91-1.39)
	1.13 (0.89-1.44)	1.05 (0.82-1.32)	1.18 (0.94-1.49)	1.10 (0.87-1.40)
	0.63 (0.51-0.78)	**0.68 (0.53-0.86)**	**0.66 (0.53-0.82)**	**0.60 (0.48-0.75)**
	1.01 (0.77-1.34)	0.88 (0.65-1.20)	1.09 (0.82-1.46)	0.90 (0.67-1.21)

Chapter 5

Added value of cardiovascular calcifications for prediction of recurrent cardiovascular events and cardiovascular interventions in patients with established cardiovascular disease

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Abstract

Background

Aim of the current study is to investigate the potential added predictive value of coronary artery calcium (CAC), thoracic aortic calcium (TAC), and heart valve calcification scores for the prediction of a combined endpoint of recurrent major cardiovascular events and cardiovascular interventions (MACE+) in patients with established cardiovascular disease (CVD).

Methods

In total, 567 patients with established CVD enrolled in a substudy of the UCC-SMART cohort were studied. Patients underwent computed tomography according to a standardized protocol and cardiovascular calcium scores were determined. Five Cox proportional hazards models for the prediction of 4-year risk of MACE+ were developed; traditional CVD risk predictors based on the SMART-risk and SMART-REACH risk scores only (model I), with addition of CAC (model II), TAC (model III), heart valve calcium (model IV), and all calcium scores (model V). Bootstrapping was performed to account for optimism. Model performance was assessed by calibration plots and discrimination (c-statistics). Additionally, the categorical net reclassification index (NRI) was evaluated.

Results

During a median follow-up time of 3.43 years (IQR 2.28-4.74) 77 events occurred for the combined endpoint MACE+. Calibration of predicted versus observed 4-year risk for model I without calcium scores was good, and the c-statistic was 0.65 (95%CI 0.59-0.72). Calibration for models II-V with addition of calcium scores was similar to model I, and c-statistics were 0.67, 0.65, 0.65, and 0.68 for model II, III, IV, and V, respectively. NRIs showed an improvement in risk classification by model II with CAC scores (NRI 15.24% (95%CI 0.59-29.39)) and for model V with all calcium scores (NRI 20.00% (95%CI 5.59-34.92)), but no improvement for models III and IV.

Conclusion

In patients with established CVD, addition of CAC scores improved performance of a risk prediction model based on classical risk factors, for the prediction of the combined endpoint MACE+. Addition of TAC or heart valve scores did not improve risk predictions.

Introduction

Cardiovascular calcification scores are related to the risk of incident cardiovascular disease, independent of traditional cardiovascular risk factors.[1-3] Whether calcification is merely a consequence of the atherosclerotic process, or that it is causally related to cardiovascular disease (CVD), is debated.[4,5] Intimal calcium deposition is even hypothesized to act as a plaque stabilizer, preventing acute atherosclerotic events.[5] Regardless of which hypothesis may hold its premise, calcium scores can be regarded as a marker of total plaque burden,[6] and could thereby reflect an individual's risk of developing cardiovascular disease.

In patients without established cardiovascular disease, addition of coronary artery calcium scores to risk prediction models provides more accurate risk predictions,[7-9] with improvements in c-statistics ranging from 0.05 to 0.13 and reported net reclassification index (NRI) ranging from 14 to 25%.[7] Furthermore, current guidelines for primary prevention recommend to consider CAC scoring in patients with predicted 10-year risk of fatal cardiovascular disease around 5% or 10% thresholds, in order to reclassify patients and thereby aid in decision making regarding preventive treatment.[10] In addition to coronary artery calcium scores and traditional risk factors, thoracic aorta calcification scores did not improve risk prediction of all-cause mortality and cardiovascular events during a mean follow-up of 8.0 (±1.5) years,[11] and neither did extra-coronary artery calcium scores, including thoracic aortic calcification, aortic valve and mitral annulus calcification for the prediction of stroke during a median follow-up of 12.1 years[12] in patients without cardiovascular disease.

In secondary prevention, patients with established cardiovascular disease are, on average, classified as high to very high risk patients.[10] However, distribution of predicted 10-year risk of recurrent cardiovascular disease risk varies widely in these patients.[13] Risk prediction models to estimate the risk of recurrent cardiovascular disease in these patients are available,[14,15] and can provide basis for intensifying treatment and give accurate prognostic information for patients. Furthermore, particularly in patients with established cardiovascular disease, calcium scores are often available as CT-imaging of the chest is often performed in these patients for various diagnostic indications.

The aim of the current study is to investigate the potential added predictive value of coronary artery, thoracic aorta, and heart valve calcification scores, on top of classical risk factors, for the prediction of a combined endpoint of recurrent major cardiovascular events (MACE) and cardiovascular interventions in patients with established cardiovascular disease.

Methods

Study population

Patients originated from a subcohort of the Utrecht Cardiovascular Cohort-Second Manifestation of ARTerial disease (UCC-SMART) cohort. The UCC-SMART cohort is an ongoing prospective cohort study starting from 1996, including 18 to 79-year-old patients referred to the University Medical Center Utrecht (UMCU), the Netherlands, with vascular disease or marked risk factors. Focus of the study is to gain insight in occurrence and risk factors of (recurrent) arterial disease in a high-risk population. Study design and rationale have been described in detail previously.(14) From August 2012, patients enrolled in the UCC-SMART cohort were invited to participate in the subcohort, consisting of cardiac non-contrast enhanced computed tomography (CT) and computed tomography angiography (CTA) of the heart, and the carotids to the circle of Willis. Exclusion criteria were known allergy to iodine containing contrast, reduced renal function (estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m2), previous exposure to CT radiation for scientific purposes, or any other contra-indications for contrast enhanced CT. The institutional board of the UMCU approved the study and all participants provided written informed consent. For the current study, 567 patients with established vascular disease at baseline enrolled in the UCC-SMART substudy with CT imaging were included. Definitions of predictors and the endpoint recurrent cardiovascular events and cardiovascular interventions are described in detail in Appendix 1.

CT-scan protocol and image analysis

Images were acquired using a 256-slice MDCT-scanner (iCT, Philips Healthcare, the Netherlands). Appendix 2 provides detailed information on the CT-scan protocol and image analysis. Non-contrast enhanced cardiac CT-scan, as well as coronary CT angiography images were acquired. Scoring of calcification spots was performed on the non-contrast enhanced cardiac CT images visualizing heart base to the pulmonary artery bifurcation. Lesions were identified by a single observer who was trained by an experienced radiologist and blinded for patient characteristics and patient outcomes. CAC was scored using the Agatston method.[16] Calcifications on heart valves and in the thoracic aorta were quantified using a pseudo-mass score, calculated by multiplying the mean calcium HU value by the region of interest (ROI) volume for every lesion, and summing up the scores of all the lesions. The thoracic aorta calcium score was comprised of the sum of the calcium scores of ascending and descending aorta. The heart valve calcium score consisted of the sum of the aortic valve and mitral annulus calcium scores. More detailed description of CT-scan protocol and image analysis is described in Appendix 2.

Incident cardiovascular events or cardiovascular interventions

During follow-up, participants received biannual questionnaires to gain information on occurrence of recurrent cardiovascular disease, bleeding events, incident diabetes, end stage renal disease, and hospitalizations for cardiovascular interventions. Additional information

was gathered by acquiring data from hospitals and general practitioners. All incident major cardiovascular events were independently judged by three physicians from an endpoint committee and conflicting classifications were resolved in consensus. Experienced research nurses adjudicated all cardiovascular interventions. Outcome of the current study was MACE+, a combined endpoint of recurrent MACE and cardiovascular interventions. MACE was defined as non-fatal myocardial infarction, non-fatal stroke or vascular death. Cardiovascular interventions included percutaneous or surgical revascularization interventions, including carotid endarterectomy (CEA), percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), major amputations, and peripheral artery stenting, angioplasty or bypass. Outcome definitions are described in detail in Appendix 1.

Predictor selection and data preparation

Predictors were selected based presence in both the original 10-year SMART-risk score[14] and the lifetime SMART-REACH risk score[15] (Appendix 3). These risk models were previously developed and externally validated to estimate the risk of recurrent cardiovascular events in patients with clinically manifest CVD.[13-15] Number of locations of vascular disease was limited to two categories (instead of three); 1 or >1 due to the low number of patients with >2 locations of vascular disease (N= 6), resulting in the following eight predictors: age, sex, current smoking (yes/no), history of diabetes mellitus (yes/no), systolic blood pressure (mmHg), total cholesterol (mmol/L), creatinine (mmol/L), and >1 location of vascular disease (yes/no); coronary heart disease (CHD), cerebrovascular disease (CeVD), or peripheral artery disease (PAD)). No missing data was observed for the predictor variables.

Development of the prediction model with and without calcium scores

Cox proportional hazards models were developed for the combined outcome MACE+, including the pre-specified predictors in the model. Too few events were observed for recurrent MACE specifically (N= 15) to perform reliable analysis. Coronary artery, thoracic aorta, and heart valve calcium scores were added to the models separately and combined, resulting in five models: (I) clinical predictors, no calcium scores (reference model), (II) model I + CAC score, (III) model I + TAC score, (IV) model I + heart valve calcium score (aortic valve and mitral annulus), (V) model I + CAC + TAC + heart valve scores. As there were only 8 competing events (non-CVD death) during follow-up, a competing risk adjusted model[17] was not considered necessary. Continuous predictors, including the cardiovascular calcium scores, were truncated at the 1st and 99th percentile to limit the effect of outliers.[18]

Bootstrapping was implemented to correct for optimism; a preferred method above split-sample especially considering the relatively small dataset and limited number of events.[19] First, models were fitted on the full original data. Second, 1000 random bootstrap samples were drawn with replacement from the original dataset and models were refitted on each bootstrap sample.

For every bootstrap sample, the difference between the performance of the bootstrap model in the bootstrap sample and the performance of the bootstrap model in the original data was determined. The average difference represented the average optimism of the models and was used to shrink model coefficients. In the original model as well as in the bootstrap models fitted in each separate bootstrap sample, linearity of the association between continuous predictors and the outcome variable was assessed by comparing Akaike's Information Criterion (AIC)[18] of a linear, squared, and log transformation of the variable. Variables were transformed appropriately to improve robustness of the model. Proportional hazards assumptions were assessed in the original model visually by plotting the scaled Schoenfeld residuals against follow-up time and no violations were observed.

Comparison of models with and without calcium scores

Prognostic performances of the five models was evaluated following previously recommended steps.[20] First, global model fit was compared by assessing the AIC of the models with and without calcium scores. Secondly, model validation was performed by assessment of various model performance measures. The validation was performed for outcome data from 4 years of follow-up (approximation of 75% percentile of follow-up duration). The calibration plots of predicted versus observed risk were compared and the c-statistic for discrimination. C-statistics were adjusted to account for optimism by assessing model performance in 1000 bootstrap samples, with confidence intervals based on the percentile method. As c-statistics usually lack power to compare models, an additional risk reclassification test is recommended.[20,21] A categorical net reclassification index (NRI) for survival data with right censoring was calculated (R package nricens), with predetermined 4-year risk categories. These risk categories were <8%, 8-13%, 13-18%, and >18%, based on interpolation by linearly adapting 10-year risks (<20%, 20-30%, 30-40%, and >40%) to 4-year risks. As no risk thresholds for preventive treatment are known for secondary prevention, or for the combined outcome MACE+ specifically, the risk difference based NRI was additionally calculated. The cut-off value was set at 0.02, meaning that only differences in predicted probability of ≥2% contributed (corresponding to a 10-year risk of 5%). Detailed methodological description of the NRI analysis is given in Appendix 2.

Model development and validation was additionally assessed for models with presence or absence (calcium score <10) of calcification instead of continuous scores for comparison. All analyses were performed in R-Statistic Programming (version 3.5.1).

Results

Baseline characteristics are shown in table 1. Mean age was 58 (SD 9) years, and prevalence of males was 77%. History of coronary artery disease was the most prevalent type of vascular disease at baseline (72%). Median (range) calcium scores were 202 (0-3941) for coronary arteries (Agatston score), and 2 (0-1820) for thoracic aorta and 1 (0-838) for heart valves (pseudo-mass score). During a median follow-up time of 3.43 years (interquartile range (IQR) 2.28-4.74) 15 recurrent cardiovascular events occurred; 6 non-fatal strokes, 7 non-fatal myocardial infarctions, and 2 vascular deaths. For the combined endpoint MACE+ (counting the first event), 77 events were observed; 5 non-fatal strokes, 6 non-fatal myocardial infarctions, 2 carotid artery interventions, 49 cardiac interventions (5 CABG and 44 PCI), 14 peripheral artery and abdominal aortic interventions, and 1 vascular death.

Development of models with and without calcium scores

AIC of the model without calcium score was 851. AIC was lower, showing a better model fit, for model II with CAC score (AIC 846) and model V with all calcium scores (AIC 848). Thoracic aorta calcium and valve calcium scores did not improve model fit according to the AIC (AIC 853 for model III and AIC 853 for model IV respectively). Model coefficients of models I, II, III, IV, and V are shown in Appendix 4. Truncated and log-transformed CAC scores were statistically significantly related to the outcome MACE+ (HR 1.53; 95%CI 1.11-2.14). Truncated (and log-transformed for valve calcium scores) scores of heart valves and thoracic aorta showed no statistically significant relation with the outcome.

Discrimination and calibration

Performance of the models was assessed by comparing calibration and discrimination. Figure 1 shows calibration plots of the predicted versus observed 4-year risk of MACE+ for the different models. Model I without calcium scores (Figure 1A) shows good calibration. Models with CAC (Figure 1B), TAC (Figure 1C), valve calcium (Figure 1D), and all calcium scores (Figure 1E) show similar calibration and no clear improvement compared to the calibration of model I. Optimism corrected c-statistics were 0.65; 95%CI 0.59-0.72 for model I without calcium scores, 0.67; 95%CI 0.61-0.73 for model II with CAC, 0.65; 95%CI 0.59-0.72 for model III with TAC, 0.65; 95%CI 0.59-0.72 for model IV with valve calcium, and 0.68; 95%CI 0.62-0.74 for model V with all calcium scores.

Table 1. Baseline characteristics

Total, N= 567	
Male, n (%)	441 (77%)
Age (years)*	58 ± 9
Current smoking, n (%)	143 (25%)
Number of pack-years*	9 (0 - 24)
Medical history	
Cerebrovascular disease (CeVD), n (%)	165 (29%)
Coronary heart disease (CHD), n (%)	408 (72%)
Peripheral artery disease (PAD), n (%)	29 (5%)
Multifocal vascular disease (eg. CHD and PAD), n (%)	52 (9%)
Diabetes Mellitus, n (%)	63 (11%)
Physical examination and laboratory measurements	
Body Mass Index (kg/m²)*	27 ± 4
Systolic blood pressure (mmHg)*	129 ± 15
Diastolic blood pressure (mmHg)*	78 ± 9
Triglycerides (mmol/L)*	1.3 (1.0 - 1.8)
Total cholesterol (mmol/L)*	4.4 ± 1.1
HDL-cholesterol (mmol/L)*	1.2 (1.0 - 1.4)
Hs-CRP (mg/L)*	1.4 (0.7 - 3.3)
eGFR (CKD-EPI, mL/min/1.73m²)*	89 ± 13
Medication	
Lipid lowering medication, n (%)	482 (85%)
Blood pressure lowering agents, n (%)	457 (81%)
Anti-platelet therapy, n (%)	499 (88%)
Anti-coagulants, n (%)	37 (7%)
Cardiovascular calcium scores	
Thoracic aorta calcium score**	2 (0 - 1820)
Coronary artery calcium score**	202 (0 - 3941)
Aortic valve and mitral annulus calcium score**	1 (0 - 838)

* Data are means ± SD or median (interquartile range). eGFR = estimated glomerular filtration rate
** Coronary artery calcium score is Agatston score. Thoracic and valve calcium scores are pseudo mass scores. Median (range) is given.

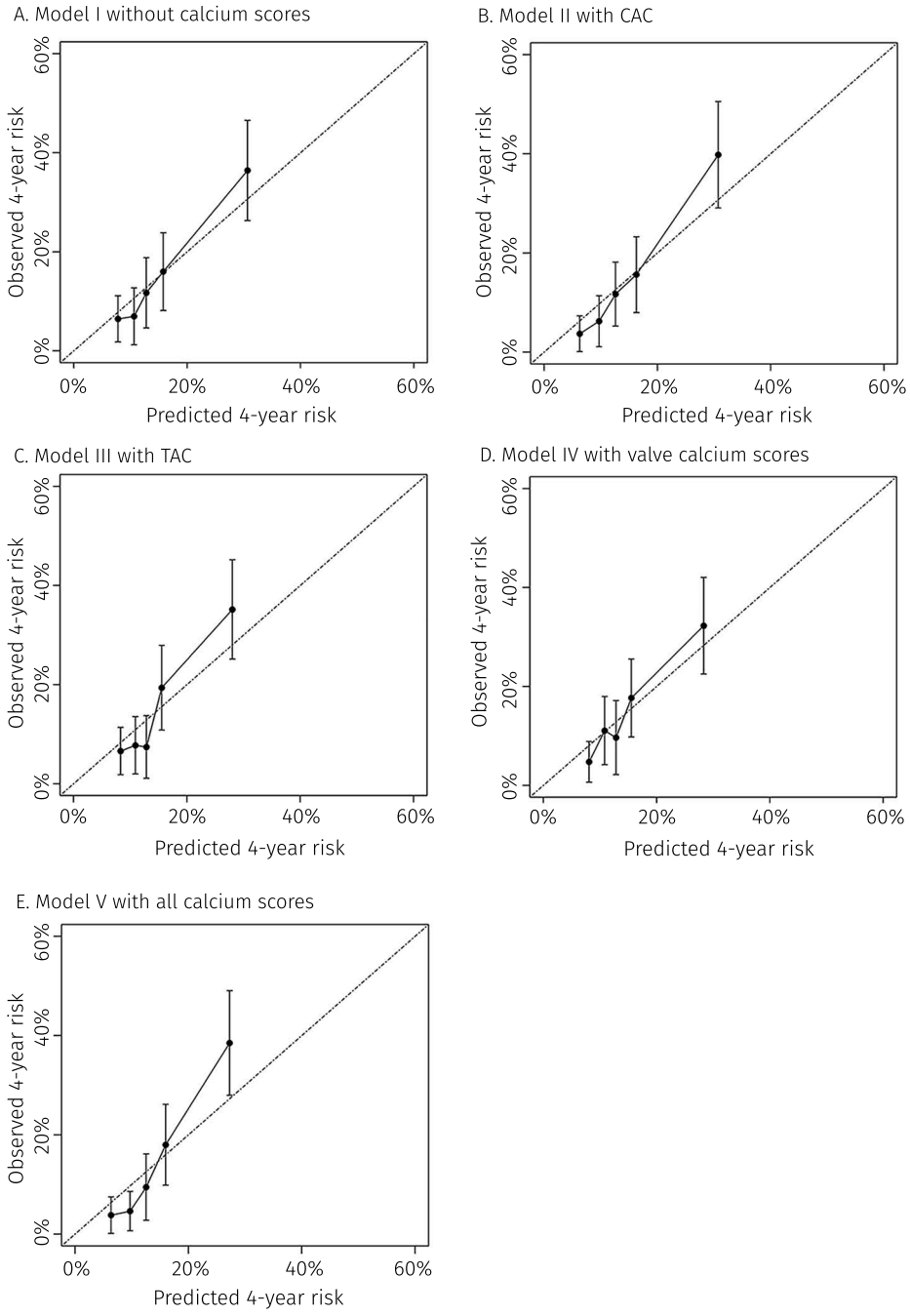

Figure 1. Calibration plots of models without and with calcium scores for the prediction of MACE+

Net reclassification index

Model II with addition of CAC scores generally reclassified patients correctly to a higher or lower risk category, according to the categorical NRI: 15.24%; 95%CI 0.59-29.39 (Table 2). Especially in patients without an events, model II reclassified patients to a lower risk category (reclassification index 8.93%; 95%CI 2.98-15.03 in the group of patients without event). Model V with all calcium scores also improved risk reclassification, as shown by a categorical NRI of 20.00%; 95%CI 5.59-34.92. Models III-V with addition of TAC scores, valve calcium scores or all calcium scores did not improve risk category classification. For the risk difference based NRI, similar results were observed with improvement in risk classification by model II with CAC scores (24.76%; 95%CI 5.10-43.60), but no improvement for models III-V) (Appendix 5). Figure 2 shows scatterplots of predicted probabilities based on the original model versus the predicted probabilities based on the expanded models including calcium scores, with symbols for patients with an event, patients without an event, and censored subjects. Model II with CAC scores and model V with all calcium scores show differences in predicted risks comparing the expanded model with the reference model without calcium scores (Figure 2 A and D). For model III with TAC and IV with valve calcium scores (Figure 2 B and C), the expanded models hardly changed risk predictions, as both patients with and patients without an event are situated along the diagonal.

Performance of models with addition of a calcification predictor indicating presence or absence of calcium (instead of continuous scores) showed similar results (model coefficients, calibration plots, c-statistics, and NRI in Appendix 6, 7, and 8).

Table 2. Categorical net reclassification index comparing models with calcium scores to model I without calcium scores for the prediction of MACE+

	Categorical reclassification index* (%)		
	With event (95% CI)	Without event (95% CI)	Net (95% CI)
Model I No scores	ref	ref	ref
Model II CAC score	6.31 (-6.23-18.56)	**8.93** **(2.87-15.03)**	**15.24** **(0.59-29.39)**
Model III TAC score	0.10 (-5.44-5.92)	**-3.45** **(-6.73- -0.19)**	-3.34 (-9.97- 3.95)
Model IV Valve scores	-5.29 (-12.54-1.06)	1.21 (-2.60-4.76)	-4.08 (-12.35-3.39)
Model V All scores	9.25 (-4.60-23.31)	**10.76** **(4.93-23.31)**	**20.00** **(5.59-34.92)**

* Categories for the categorical were based on 10-year risk categories <20%, 20-30%, 30-40%, and >40% translated to 4-year risks: <9%, 9-13%, 13-18%, >18%.

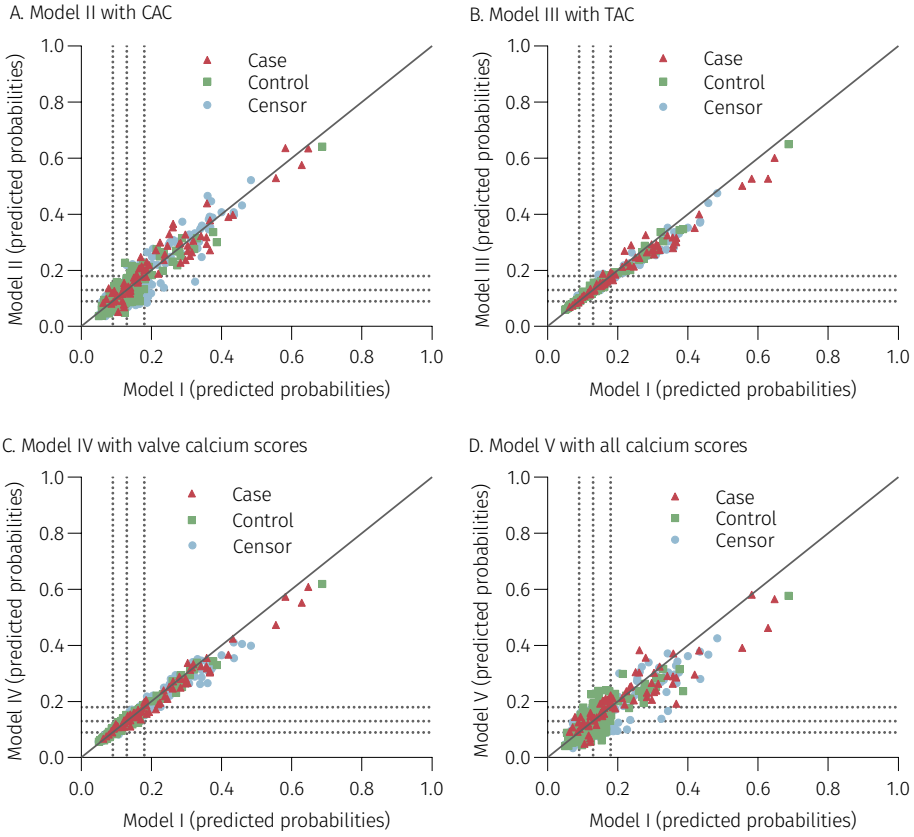

Figure 2. Predicted probabilities for patients with an event and patients without an event by models with calcium scores compared to model I without calcium scores for the prediction of MACE+

The diagonal line added to the plot indicates no change in the predicted probabilities. If the expanded prediction model improved reclassification, events will lie above the diagonal (higher predicted probability with the new model) and will have switched to a higher risk category, whereas controls will appear below the diagonal (lower predicted probability with the new model) and will have switched to a lower risk category. The dotted lines represent the 4-year risk thresholds: 9%, 13%, and 18%.

Discussion

The present study shows that in patients with established cardiovascular disease, addition of CAC scores to a prediction model with classical atherosclerotic risk factors for estimating the risk of MACE+, provides similar calibration and discrimination, and improves global model fit and risk classification for 4-year risk predictions. Addition of TAC or heart valve calcium scores did not improve measures of model performance.

Extra-coronary thoracic cardiovascular calcification scores, including thoracic aorta and heart valves, did not improve risk predictions in the current study, in accordance with previous studies in patients without established cardiovascular disease.[11,12] Addition of CAC scores did improve risk predictions in the current study, in terms of global model fit and risk reclassification. Similar calibration and c-statistics were observed for the model with CAC scores in comparison to the model with only traditional risk factors. However, c-statistics often lack statistical power to compare models, and conclusions should not be solely based on this model performance measure.[20,21] Although calibration, an important measure for prognostic risk model performance,[20] was similar, the NRI showed that addition of CAC scores to a prediction model with traditional risk factors correctly reclassified patients to a higher or lower risk category. Therefore, CAC scores were considered of additional prognostic value for the prediction of MACE+ in patients with established cardiovascular disease. These results are also in accordance with previous studies in apparently healthy people where addition of CAC scores to models with classical risk factors was found to improve model performance.[7,8,22-26] Furthermore, a relation between CAC scores and the combined endpoint MACE+ in patients with stable CVD was previously observed, with a HR of 1.35; 95%CI 1.15-1.58 (per SD higher calcium score),[27] and between CAC>0 and MACE in patients with suspected CHD with a pooled relative risk ratio of 5.71; 95%CI 3.98-8.19.[3]

Coronary artery calcification can be regarded as a measure of total plaque burden,[6] and in that capacity calcium scores will provide additional prognostic value for the prediction of (recurrent) cardiovascular events. The process of coronary artery calcification is thought to act as a plaque stabilizer,[5] as large dense calcification spots of more than 400 Hounsfield Units (HU) are commonly associated with stable plaques and microcalcifications of lower density with instable, vulnerable plaques.[5,28] Furthermore, long term and high dose statin use is thought to accelerate coronary plaque calcification without leading to more frequent cardiovascular events,[29] suggesting that CAC increase under statin treatment represents plaque stabilization rather than plaque expansion.[29-31] As was previously shown in patients without established CVD,[32-35] markers of calcification morphology or stenosis severity, undistinguished by the CAC score, potentially provide additional prognostic value for risk prediction beyond CAC scores in patients with established CVD, and this will be investigated in future work.

Primary prevention guidelines recommend to use available CAC scores in patients with predicted risks around 5% or 10% risk factor treatment thresholds for risk reclassification.[10] Furthermore, the ongoing 'risk or benefit in screening for cardiovascular diseases' (ROBINSCA) trial, a large-scale population-based cardiovascular disease screening trial, is investigating the impact of CAC imaging and subsequent preventive treatment on CVD morbidity and mortality in apparently healthy people.[36,37] As the present study showed that CAC scores improve risk estimations of MACE+ in patients with established cardiovascular disease, implementation of available CAC scores in risk prediction could be recommended. Particularly in patients with established CVD, CAC scores are often available as CT-imaging of the chest is often performed for various diagnostic indications in these patients. Although no risk thresholds for preventive treatment are available for secondary prevention, accurate risk predictions could lead to justified treatment intensification or downgrading. Novel and costly therapies are available, such as PCSK9 inhibitors,[38] and new antithrombotic treatment schemes, such as dual antiplatelet therapy or adding rivaroxaban to aspirin (dual pathway inhibition),[39] aiming to reduce cardiovascular disease risk. Accurate risk predictions are needed to distinguish patients with the highest risk that will benefit the most from these novel therapies. Currently, the SMART risk score[14] and the SMART-REACH model[40] are the standard for 10-year and lifetime predictions, respectively, of recurrent cardiovascular events in patients with established cardiovascular disease. Although these models performed well in external validation,[13,40,41] further improvement could enhance prediction accuracy. In future studies, risk prediction models could be developed with addition of CAC scores as extensions to the SMART-risk and SMART-REACH model, for patients with available CAC scores. These models could estimate the risk of MACE+, and potentially CAC scores are also of added prognostic value for the prediction of MACE specifically. Since the simple model with CAC absence or presence performed similarly compared to the model with continuous CAC scores, it might be considered to develop models with continuous CAC scores as well as a simple presence or absence score, in order to benefit the most of available information for cardiovascular risk prediction in patients with established cardiovascular disease.

The present study has several strengths, including the cohort of patients with established cardiovascular disease with available cardiovascular calcium scores and follow-up data. Furthermore, several model performance measures were evaluated, including discrimination, calibration, and NRI. Limitations should be considered and include the limited length of follow-up. Therefore, validation of the models could only be performed for 4-years of follow-up. Furthermore, due to the limited number of events, reliable analyses of specific recurrent cardiovascular events (recurrent MACE specifically, stroke or coronary artery disease), or subgroup analyses could not be performed. As the majority of the study population had a history of coronary heart disease, subgroup analyses in patients with cerebrovascular or peripheral artery disease specifically would strengthen generalizability to all patients with established cardiovascular disease. As the number of events (N=77) is limited, and does not

reach the recommended minimum number of 100 events for validation of a prediction model,[42,43] the results of the current study are preliminary findings for a population with established cardiovascular disease, for whom to our knowledge no previous studies were specifically performed to assess the added prognostic value of calcium scores. Additionally, calcification lesions were not assessed by a second observer. However, all extra-coronary lesions were scored by one individual, thereby limiting between-image variability. Lastly, models were internally validated potentially leading to optimism. Overestimation of the performance of model I without calcium scores could lead to a potential underestimation of performance improvements by models with calcium scores. However, discrimination and calibration were adjusted for optimism by bootstrapping, limiting this effect.[44]

In conclusion, in patients with established CVD, addition of CAC scores improved performance of a risk prediction model based on classical risk factors, for the prediction of the combined endpoint MACE+. Addition of TAC or heart valve scores did not improve risk predictions.

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Appendices

Appendix 1. Predictor and outcome definitions in the UCC-SMART cohort

A. Definition of baseline characteristics [1,2]	
Age	Years, reported by physician/patient
Sex	Male/female, reported by physician/patient
Current smoking	Current vs never/former, reported by patient
History of diabetes mellitus	Either referral diagnosis, self-reported, or a known history of diabetes mellitus at the time of enrolment or a fasting blood glucose ≥7 mmol/L.
Systolic blood pressure	mmHg. Measured directly after informed consent. Mean of two office blood pressure measurements.
Total cholesterol	mmol/L. Measured in fasting venous sample using commercial enzymatic dry chemistry kits (Johnson and Johnson)
Creatinine	µmol/L. Measured in fasting venous sample using commercial enzymatic dry chemistry kits (Johnson and Johnson).
History of peripheral artery disease	Symptomatic and documented obstruction of distal arteries of the leg of surgery of the leg (percutaneous transluminal angioplasty, bypass or amputation)
History of coronary heart disease	Angina pectoris, myocardial infarction or coronary revascularization (coronary bypass surgery or coronary angioplasty)
History of cerebrovascular disease	TIA, cerebral infarction, amaurosis fugax or retinal infarction, or a history of carotid surgery

B. Definition of recurrent cardiovascular disease [1,2]	
Non-fatal myocardial infarction	At least two of the following: 1. Chest pain for at least 20 minutes, not disappearing after administration of nitrates; 2. ST-elevation >1 mm in two following leads or a left bundle branch block on the ECG; 3. CK elevation of at least two times the normal value of CK and a MB-fraction >5% of the total CK.
Non-fatal stroke	Relevant clinical features which have caused an increase in handicap of at least one grade on the modified Rankin scale, accompanied by fresh infarct or hemorrhage on a repeat CT scan
Vascular death	Sudden death (unexpected cardiac death occurring within 1 hour after onset of symptoms, or within 24 hours given convincing circumstantial evidence) or death from stroke, myocardial infarction, congestive heart failure, rupture of abdominal aortic aneurysm, or from other cause, i.e. sepsis following stent placement.

Appendix 1. Continued

C. Definition of vascular intervention [1,2]*	
Heart	Percutaneous coronary intervention or coronary artery bypass surgery
Carotid artery or intracranial arteries	Stent, angioplasty, (thrombo)endarterectomy, bypass surgery
Peripheral	Stent or graft (endovascular or open surgery), angioplasty, bypass surgery or (thrombo)endarterectomy. Major amputation (excluding toe or forefoot amputation[3]) due to arterial ischemia. Other intervention due to ischemia (eg emergency laparotomy due to intestinal ischemia, urokinase treatment, surgical correction of endoleak after EVAR, nephrectomy due to atherosclerotic cause, kidney transplantation)

* Patients who received a vascular intervention in response to a cardiovascular disease event, are classified according to the cardiovascular disease event

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Appendix 2. Supplemental methods

CT-scan protocol

Non-contrast enhanced cardiac CT-scans were ECG gated with a standard tube voltage of 120 peak kilovoltage (kVp). To achieve most optimal imaging for the coronary CTA, patients whose heart rate exceeded 70 bpm were given beta-adrenergic blocking medication. A weight-dependent bolus of 70-90 ml iodine contrast was injected. Coronary CT angiography was performed using prospectively triggered protocols synchronized to the diastolic resting phase (78% of the RR-interval). In patients in whom the heart rate could not be lowered to 70 beats or less minute, or in cases of irregular heart rate retrospective gating was used.

Image analysis

For 2 patients coronary artery calcification score was missing. For 7 patients, a non-contrast enhanced cardiac scan was absent, due to missing thin slice reconstruction, interfering artefacts, or technical problems. Patients with aortic valve replacement (n=9) or mitral valve replacement (n=6) were excluded from the analysis with heart valve calcification scores. Therefore, final study population numbers were 565 for development and validation of model II (with CAC), 560 for model III (with TAC), 546 for model IV (with valve calcium scores), and 545 for model V (with all calcium scores). Thin slice reconstructions were created for scoring thoracic aorta and cardiac valve calcification with a slice thickness of 0.9 mm at 0.45 mm increment. For coronary arteries a slice thickness of 3.0 mm at 3.0 mm increment was used according to Agatston guidelines.[1]

Calcification lesions were identified by the observer and the dedicated software (iX Viewer, ImageSciences Institute, Utrecht, the Netherlands) automatically selected calcifications across consecutive slices. The automatic selection was inspected per slice and manually corrected if necessary, to prevent overestimation. Calcification in the aortic root was considered part of the aortic valve.[2] If a calcification lesion occupied multiple anatomical locations, it was scored according to its predominant location. For the selection of calcification on the non-contrast enhanced scans a standard threshold of 130 Hounsfields Units (HU) was implemented.

Data analysis

Net reclassification index

The continuous net reclassification index (NRI) is calculated by assessing the change in predicted probability for cases and controls comparing the base model to models with an additional predictor, and is defined as: NRI = P(up|event) - P(down|event) + P(down|nonevent) - P(up|event). Originally, the NRI was developed for binary outcome and did not handle censoring.[3,4] A prospective form of NRI has been proposed, allowing for NRI calculations in survival data with right censoring.[5] The new formulation of the NRI can be interpreted as a measure of event rate increase among those who are reclassified upwards, and event rate decrease among those

who are reclassified downwards, with event rates estimated by the Kaplan-Meier approach.[5] The reclassification can graphically be displayed by plotting predicted probabilities based on the original model versus the predicted probabilities based on the extended model, with symbols for cases and controls. The diagonal line added to the plot indicates no change in the predicted probabilities. If the extended prediction model improved reclassification, events will lie above the diagonal (higher predicted probability with the new model), whereas controls will appear below the diagonal (lower predicted probability with the new model).[5,6]

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Appendix 3. Predictor selection based on presence in both SMART-REACH model and SMART-risk score

SMART-REACH model[1]	SMART-risk score[2]	Final selection for current study
Age (years)	Age (years)	Age (years)
Sex (male/female)	Sex (male/female)	Sex (male/female)
Current smoking (yes/no)	Current smoking (yes/no)	Current smoking (yes/no)
Diabetes mellitus (yes/no)	Diabetes mellitus (yes/no)	Diabetes mellitus (yes/no)
Number of locations of vascular disease (1, 2, or 3)	Coronary heart disease, cerebrovascular disease, peripheral artery disease, abdominal aorta aneurysm as 4 separate predictors	>1 location of vascular disease
Systolic blood pressure (mmHg)	Systolic blood pressure (mmHg)	Systolic blood pressure (mmHg)
Total cholesterol (mmol/L)	Total cholesterol (mmol/L)	Total cholesterol (mmol/L)
Creatinine (µmol/L)	Estimated glomerular filtration rate (ml/min/1.73 m^2)	Creatinine (µmol/L)
Atrial fibrillation (yes/no)		
Congestive heart failure (yes/no)*		
	HDL cholesterol (mmol/L)	
	C-reactive protein (mg/L)	
	Years since first vascular event	

* Not available in UCC-SMART data

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Appendix 4. Hazard ratios, 95% confidence intervals, and shrinkage factor of models with and without calcium scores

A. Model I (No calcium scores)

	Coefficient	HR	95% CI lower limit	95% CI upper limit
Age	0.0495	1.0507	0.8167	1.3517
Creatinine	-0.0675	0.9347	0.7231	1.2082
Sex	0.7159	2.0461	0.9596	4.3624
Systolic blood pressure	0.2138	1.2384	0.9927	1.5448
Total cholesterol	0.1631	1.1771	0.9182	1.5091
Current smoking	0.0567	1.0584	0.6208	1.8043
Diabetes mellitus	1.0864	2.9636	1.7546	5.0056
>1 location of vascular disease	1.1180	3.0588	1.7672	5.2943

Shrinkage factor 0.8278. For continuous predictors, hazard ratios per 1SD

B. Model II (coronary artery calcium score)

	Coefficient	HR	95% CI lower limit	95% CI upper limit
Age	-0.1144	0.8919	0.6706	1.1861
Creatinine	-0.0321	0.9684	0.7510	1.2488
Sex	0.4839	1.6224	0.7499	3.5101
Systolic blood pressure	0.2012	1.2229	0.9786	1.5283
Total cholesterol	0.1937	1.2137	0.9450	1.5589
Current smoking	0.0388	1.0396	0.6091	1.7743
Diabetes mellitus	1.0787	2.9409	1.7453	4.9556
>1 location of vascular disease	0.9636	2.6211	1.5003	4.5792
log(CAC score)	0.4276	1.5336	1.1006	2.1370

Shrinkage factor 0.8037. For continuous predictors, hazard ratios per 1SD

Appendix 4. Continued

C. Model III (thoracic aorta calcium score)

	Coefficient	HR	95% CI lower limit	95% CI upper limit
Age	0.0212	1.0214	0.7802	1.3372
Creatinine	-0.1183	0.8884	0.6825	1.1564
Sex	0.7554	2.1284	0.9968	4.5448
Systolic blood pressure	0.1764	1.1929	0.9464	1.5036
Total cholesterol	0.1488	1.1604	0.9023	1.4924
Current smoking	0.0794	1.0827	0.6343	1.8479
Diabetes mellitus	0.9670	2.6300	1.5097	4.5816
>1 location of vascular disease	1.0823	2.9516	1.6711	5.2133
TAC score	0.0660	1.0683	0.8824	1.2932

Shrinkage factor 0.7704. For continuous predictors, hazard ratios per 1SD

D. Model IV (valve calcium scores)

	Coefficient	HR	95% CI lower limit	95% CI upper limit
Age	0.1229	1.1308	0.8407	1.5209
Creatinine	-0.1246	0.8829	0.6752	1.1544
Sex	0.7873	2.1974	1.0201	4.7336
Systolic blood pressure	0.1646	1.1789	0.9322	1.4910
Total cholesterol	0.1555	1.1683	0.9054	1.5076
Current smoking	0.0588	1.0606	0.6097	1.8449
Diabetes mellitus	1.0888	2.9707	1.7055	5.1744
>1 location of vascular disease	1.1771	3.2450	1.7941	5.8692
log(valves calcium score)	-0.1111	0.8948	0.6847	1.1694

Shrinkage factor 0.7791. For continuous predictors, hazard ratios per 1SD

Appendix 4. Continued

E. Model V (all calcium scores combined)

	Coefficient	HR	95% CI lower limit	95% CI upper limit
Age	-0.0292	0.9712	0.6972	1.3528
Creatinine	-0.0975	0.9071	0.6948	1.1843
Sex	0.5476	1.7291	0.7968	3.7519
Systolic blood pressure	0.1517	1.1638	0.9168	1.4774
Total cholesterol	0.1998	1.2212	0.9437	1.5801
Current smoking	0.0506	1.0519	0.6028	1.8357
Diabetes mellitus	1.0163	2.7631	1.5703	4.8618
>1 location of vascular disease	0.9862	2.6809	1.4455	4.9722
log(valves calcium score)	-0.2045	0.8151	0.6178	1.0752
TAC score	0.0544	1.0559	0.8614	1.2943
log(CAC score)	0.4953	1.6410	1.1580	2.3254

Shrinkage factor 0.7277. For continuous predictors, hazard ratios per 1SD

Appendix 5. Risk difference based net reclassification index comparing models with calcium scores to model I without calcium scores for the prediction of MACE+

	Risk difference based reclassification index (%)		
	With event (95% CI)	Without event (95% CI)	Net (95% CI)
Model 1 No scores	ref	ref	ref
Model II CAC score	14.74 (-3.16-31.46)	**10.01** **(2.82-17.07)**	**24.76** **(5.10-43.60)**
Model III TAC score	**-19.45** **(-30.80- -7.73)**	**6.67** **(3.59-9.77)**	**-12.78** **(-24.33- -0.53)**
Model IV Valve scores	**-22.04** **(-35.64- -9.26)**	**5.99** **(2.32-9.46)**	**-16.05** **(-29.68- -2.55)**
Model V All scores	-2.41 (-21.73-16.29)	**18.62** **(11.38-25.09)**	16.20 (-6.21-36.37)

A cut-off value of 2% was used for the risk difference based reclassification index

Appendix 6. Hazard ratios, 95% confidence intervals, and shrinkage factor of models with and without calcium presence/absence for the prediction of recurrent MACE and cardiovascular interventions

A. Model II (coronary artery calcium)

	HR	95% CI lower limit	95% CI upper limit
Age	0.9435	0.7200	1.2363
Creatinine	0.9585	0.7422	1.2377
Sex	1.7517	0.8132	3.7733
Systolic blood pressure	1.2042	0.9367	1.5480
Total cholesterol	1.0251	0.6005	1.7497
Current smoking	1.0251	0.6005	1.7497
Diabetes mellitus	2.8912	1.7168	4.8689
>1 location of vascular disease	2.8108	1.6210	4.8738
CAC present	2.6520	1.0846	6.4843

Shrinkage factor 0.8067. For continuous predictors, hazard ratios per 1SD

B. Model III (thoracic aorta calcium)

	HR	95% CI lower limit	95% CI upper limit
Age	0.9992	0.7507	1.3300
Creatinine	0.8919	0.6872	1.1576
Sex	2.1915	1.0219	4.6994
Systolic blood pressure	1.1938	0.9492	1.5014
Total cholesterol	1.1481	0.8916	1.4783
Current smoking	1.0694	0.6255	1.8283
Diabetes mellitus	2.6396	1.5243	4.5710
>1 location of vascular disease	3.0354	1.7467	5.2751
TAC present	1.2514	0.7131	2.1961

Shrinkage factor 0.7986. For continuous predictors, hazard ratios per 1SD

Appendix 6. Continued

C. Model IV (valve calcium)

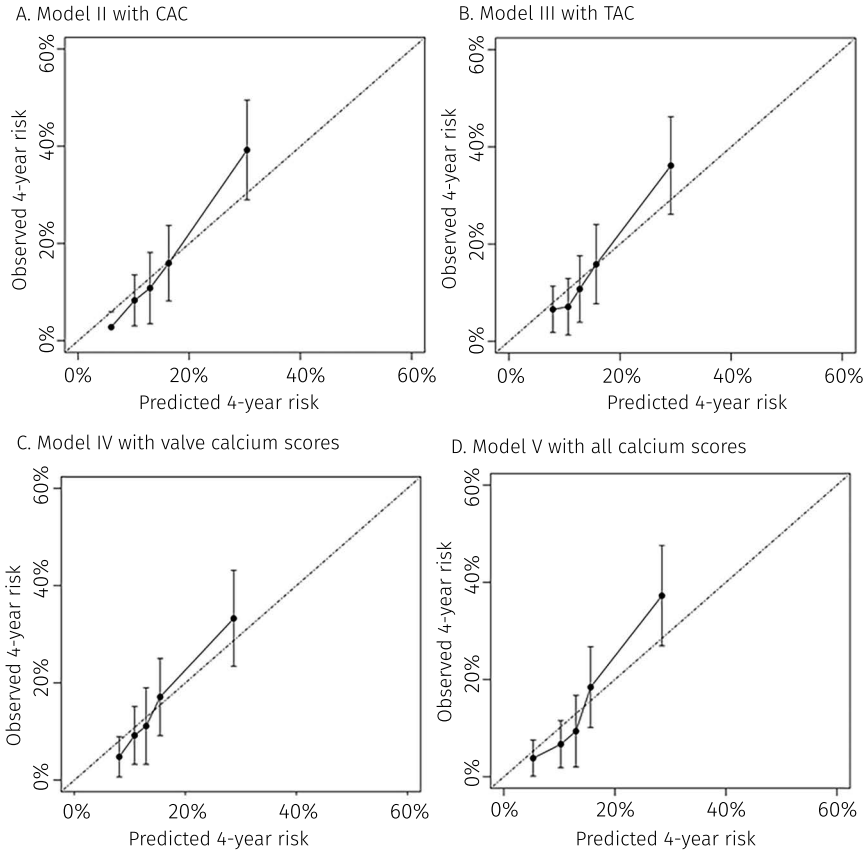
	HR	95% CI lower limit	95% CI upper limit
Age	1.0968	0.8237	1.4605
Creatinine	0.8836	0.6763	1.1544
Sex	2.1698	1.0086	4.6679
Systolic blood pressure	1.1785	0.9330	1.4885
Total cholesterol	1.1646	0.9016	1.5045
Current smoking	1.0476	0.6032	1.8194
Diabetes mellitus	2.9178	1.6814	5.0633
>1 location of vascular disease	3.1760	1.7611	5.7276
Valve calcium present	0.8717	0.5001	1.5193

Shrinkage factor 0.7945. For continuous predictors, hazard ratios per 1SD

D. Model V (all calcium presence/absence combined)

	HR	95% CI lower limit	95% CI upper limit
Age	0.9662	0.6944	1.3444
Age^2	0.9001	0.6901	1.1739
Creatinine	2.0031	0.8896	4.5105
Sex	1.1802	0.9338	1.4917
Systolic blood pressure	1.0426	0.7813	1.3912
Total cholesterol	1.2466	0.9706	1.6012
Current smoking	0.9930	0.5701	1.7296
Diabetes mellitus	2.7777	1.5762	4.8949
>1 location of vascular disease	2.7795	1.5346	5.0342
Valve calcium present	0.8463	0.4863	1.4727
TAC present	1.0424	0.5753	1.8888
CAC present	3.3719	1.2691	8.9590

Shrinkage factor 0.7644. For continuous predictors, hazard ratios per 1SD



Appendix 7. Calibration plots of models with calcium presence/absence for the prediction of MACE+

Appendix 8. C-statistics and net reclassification index for models with and without calcium presence/absence as predictor for MACE+

	C-statistics	**Net reclassification index (NRI) (%)**	
	(95%CI)	Risk difference based NRI* (95% CI)	Categorical NRI** (95% CI)
Model 1 No scores	0.65 (0.59-0.72)	ref	ref
Model II CAC score	0.68 (0.61-0.74)	**24.04 (8.26-38.83)**	**17.30 (1.98-32.31)**
Model III TAC score	0.65 (0.59-0.71)	-10.25 (-22.21- 0.82)	0.79 (-5.73-6.65)
Model IV Valve scores	0.65 (0.58-0.71)	**-14.58 (-27.11- -3.17)**	0.32 (-9.64-11.23)
Model V All scores	0.67 (0.61-0.72)	8.13 (-12.02-29.24)	12.54 (-2.32-28.53)

* A cut-off value of 2% was used for the risk difference based reclassification index

** Categories for the categorical were based on 10-year risk categories <20%, 20-30%, 30-40%, and >40% translated to 4-year risks: <9%, 9-13%, 13-18%, >18%.

Part III

Individualized risk prediction

Chapter 6

Predicting 10-year risk of recurrent cardiovascular events and cardiovascular interventions in patients with established cardiovascular disease: results from UCC-SMART and REACH

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In revision

Abstract

Background

Existing cardiovascular risk scores for patients with established cardiovascular disease estimate residual risk of recurrent major cardiovascular events (MACE). Cardiovascular interventions are also clinically relevant. The aim of the current study is to develop and externally validate a prediction model to estimate the 10-year combined risk of recurrent MACE and cardiovascular interventions, the extended SMART risk score, in patients with established cardiovascular disease.

Methods

Data of patients with established cardiovascular disease from the UCC-SMART cohort (N=8,421) were used for model development, and patient data from REACH Western Europe (N=14,528) and REACH North America (N=19,495) for model validation. Predictors were selected based on the existing SMART risk score. A Fine and Gray competing risk-adjusted 10-year risk model was developed for the combined outcome recurrent MACE and cardiovascular interventions (MACE+). The model was validated in all patients with cardiovascular disease and in strata of coronary heart disease (CHD), cerebrovascular disease (CeVD), peripheral artery disease (PAD).

Results

External calibration for 2-year risk in REACH Western Europe and REACH North America was good, c-statistics were moderate: 0.60 and 0.58, respectively. In strata of cardiovascular disease at baseline good external calibration was observed in patients with CHD and CeVD, however, poor calibration was seen in patients with PAD. C-statistics for patients with CHD were 0.60 and 0.57, for patients with CeVD 0.62 and 0.61, and for patients with PAD 0.53 and 0.54 in REACH Western Europe and REACH North America, respectively.

Conclusions

The 10-year combined risk of recurrent MACE and cardiovascular interventions can be estimated in patients with established CHD or CeVD. However, cardiovascular interventions in patients with PAD can not be predicted reliably.

Introduction

The number of patients in the chronic phase of cardiovascular disease (CVD) is growing as a result of improved survival after acute vascular events, an ageing populations, and deteriorating lifestyle habits such as sedentary behavior and unhealthy diet leading to obesity.[1-5] In order to successfully prevent a second cardiovascular event in a patient with established cardiovascular disease, preventive treatment strategies should be personalized to fit each individual patient. In particular with regard to emerging, and often costly, therapies such as proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors[6-9] intensified anti-thrombotic treatment schemes,[10,11] specific anti-inflammatory[12] or icosapent ethyl treatment,[13] it is essential to identify those patients with the highest residual cardiovascular risk, as these patients will benefit the most. Relevant for clinical practice is also that risk estimations can be used to inform patients of their prognosis and to facilitate shared decision making concerning preventive treatment.[14-16]

The SMART risk score[17] is commonly used for patients with established cardiovascular disease, for patient education and as a clinical decision-support tool. Physicians and patients can access interactive calculators of the SMART risk score in the 'ESC CVD risk calculation'-app, on the ESC-website (https://www.escardio.org/Education/ESC-Prevention-of-CVD-Programme/Risk-assessment/SMART-Risk-Score), and on U-Prevent (http://u-prevent.com). The SMART risk score predicts the 10-year residual risk of recurrent major cardiovascular events (MACE), defined as non-fatal myocardial infarction, non-fatal stroke, or vascular death.[14,17,18] Incidence rates of these events have however steadily declined by in total 53% between 1996 and 2014 in a cohort of patients with stable cardiovascular disease.[19] As this decline is only partially explained by improved treatment of risk factors,[19] it may also be due to earlier detection of atherosclerotic disease[20,21] and subsequent (preventive) cardiovascular interventions, forestalling part of the acute ischemic events. This attention to cardiovascular interventions is also evident from the results of recent cardiovascular prevention trials, which usually report the effects for a combined outcome of major cardiovascular events as well as coronary revascularizations, as secondary[8] or even primary outcome,[6] and also include peripheral interventions.[10] Most importantly, cardiovascular interventions such as amputations, peripheral revascularization procedures, cardiac interventions, and carotid endarterectomy cause significant morbidity,[22,23] and from a patient's perspective might have a similar clinical impact as classical MACE. For these reasons, calculating the risk of both cardiovascular events and cardiovascular interventions might provide a more accurate estimation of an individual's future health and risk, and provide a more appropriate translation from trial results to clinical practice, thereby aiding in determining preventive treatment strategies, informing patients, and facilitating shared decision making.

Therefore, the aim of the current study is to develop and externally validate a risk prediction model for estimating the 10-year combined residual risk of recurrent MACE and cardiovascular interventions in patients with established cardiovascular disease.

Methods

Study populations

Participants originated from the Utrecht Cardiovascular Cohort-Second Manifestations of ARTerial disease (UCC-SMART) cohort, and the REduction of Atherothrombosis for Continued Health (REACH) Registry, both prospective cohorts including patients with established cardiovascular disease or risk factors for atherosclerotic disease. Study designs and rationales have been described in detail previously.[24-32] From both cohorts, patients with established cardiovascular disease at baseline were included for the current analyses.

UCC-SMART is an ongoing prospective cohort including 18-79 year-old patients referred to the University Medical Center Utrecht (UMCU) in the Netherlands, that started enrollment in 1996 and is still recruiting. At baseline, information on medical history, and physical examination and laboratory measurements are acquired following a standardized protocol. The international REACH registry included patients between 2003 and 2004 from general practitioners or medical specialist outpatient practices from countries in North America, Latin America, Europe, the Middle East, Asia, and Australia. Medical history, physical and laboratory measurements were collected according to a standardized international case report form.[26] Definitions of baseline characteristics of the cohorts are described in detail in Appendix 1A. Both the UCC-SMART cohort and the REACH-registry were approved by an institutional review board, and written informed consent was obtained from all participants. For the current study, patients with established cardiovascular disease from UCC-SMART enrolled between September 1996 and March 2018 (N=8,421) from REACH Western Europe (N=14,528) and from REACH North America (N=19,495) were included.

Recurrent cardiovascular events and cardiovascular interventions

For the UCC-SMART cohort, information on the occurrence of recurrent MACE, bleeding events, incident diabetes, end stage renal disease, and hospitalizations for cardiovascular interventions was obtained by biannual questionnaires sent out to participants. Additional information was gathered from hospitals and general practitioners. An endpoint committee of three physicians adjudicates all recurrent cardiovascular disease events and experienced research nurses judged all cardiovascular interventions. Conflicting decisions were discussed and resolved in consensus.

Patients from the REACH registry returned for follow-up visits annually with a maximum follow-up duration of 4 years. Occurrence of recurrent cardiovascular events, hospitalization for unstable angina pectoris, congestive heart failure, major bleeding events, and cardiovascular interventions were reported by a local investigator and not adjudicated.

The endpoint for the current study was the combined outcome of recurrent MACE and cardiovascular interventions (MACE+). MACE was defined as non-fatal myocardial infarction, non-fatal stroke, or vascular death. Cardiovascular interventions included percutaneous interventions or revascularization surgery; carotid endarterectomy (CEA), percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), lower limb amputations, and peripheral artery stenting, angioplasty or bypass (overview is presented in Table 2, and detailed definitions are presented in Appendix 1B and 1C).

Temporal validation of existing SMART risk score for recurrent MACE

To evaluate the performance of the original SMART risk score from 2013 for the prediction of recurrent MACE,[17] temporal validation was performed in the larger UCC-SMART dataset (Appendix 2 for details on number of patients and events) with all patients and in strata of cardiovascular disease at baseline (coronary heart disease (CHD), cerebrovascular disease (CeVD), and peripheral artery disease (PAD)). External validation of the SMART risk score in the REACH datasets has previously been performed.[14]

Predictor selection and data preparation

For the new prediction model, i.e. the extended SMART risk score, to estimate the risk of MACE+ (recurrent MACE and cardiovascular interventions combined), the predictors were selected from the original SMART risk score. A subsequent literature search did not provide additional predictors of incident cardiovascular interventions, resulting in the following 14 predictors: age, sex, current smoking (yes/no), history of diabetes mellitus (yes/no), systolic blood pressure (mmHg), total cholesterol (mmol/L), high density lipoprotein (HDL) cholesterol (mmol/L), high sensitive C-reactive protein (CRP) (mg/L), estimated glomerular filtration rate (eGFR) (mL/1.73m2), time since first cardiovascular event (years), history of coronary heart disease (yes/no), history of cerebrovascular disease (yes/no), history of peripheral artery disease (yes/no), and history of aneurysm of the abdominal aorta (yes/no). Missing data (≤1% per variable in UCC-SMART, and in REACH 18% for kidney function, 17% for total cholesterol, 2% for current smoking, and <1% for other variables) was singly imputed by predictive mean matching based on multivariable regression using both baseline and outcome data (aregImpute function in R, Hmisc package). Continuous predictors were truncated to the 1st and 99th percentile to limit influence of outliers (continuous predictors in the REACH datasets were truncated to the limits of these variables in UCC-SMART).

Model development for estimating risk of MACE+

A Fine and Gray competing risk-adjusted subdistribution hazard function[33,34] was developed in the UCC-SMART cohort for 10-year predictions. Non-cardiovascular death was considered the competing endpoint. Because of the longer follow-up period, the UCC-SMART dataset was preferred as derivation cohort. To improve the model fit, log and quadratic associations

between continuous predictors and the outcome variable were assessed by comparing Akaike's Information Criterion (AIC),[35] and transformations were applied when appropriate. The proportional hazards assumption was assessed visually by plotting scaled Schoenfeld residuals and no violations were observed. The linear predictor was adjusted by a shrinkage factor, acquired by bootstrapping with a 1000 bootstrap samples, to account for optimism.

External validation in REACH Western Europe and REACH North America

External validation of the extended SMART risk score was performed in REACH Western Europe and REACH North America. As the predictors CRP, HDL cholesterol, and time since first cardiovascular event were not available in the REACH dataset, population averages of UCC-SMART were imputed for these variables. This method is preferred over excluding the predictor and performs similar compared to subgroup mean imputation and multiple imputation if the predictor is less important.[36] Model performance was assessed by the c-statistic for discrimination and calibration plots of predicted versus observed risks. The validation was performed for outcome data from 2 years of follow-up (approximation of median follow-up time), by implementing the 2-year baseline hazard from the derivation dataset (UCC-SMART) and using the same coefficients that were determined in the derivation set during model development. To adjust for variation in the underlying event rates, the expected observed ratio in the REACH Western Europe and the REACH North America study populations was used to recalibrate the model. Additionally, the risk score was validated in patients from REACH Western Europe and REACH North America in strata of cardiovascular disease at baseline (CHD, CeVD, and PAD) with the previously determined expected observed ratios. For the current study, abdominal aortic aneurysm (AAA) was not included in the definition of PAD.

All analyses were performed with R statistical software (version 3.5.1). To enable the use of this newly developed risk model in daily clinical practice, an online calculator will be developed that allows estimation of 10-year risk of MACE+ for an individual patient.

Results

Baseline characteristics and number of recurrent MACE and cardiovascular interventions

Baseline characteristics of patients in UCC-SMART, REACH Western Europe, and REACH North America are presented in Table 1. In the REACH cohorts, patients were generally older, with a mean age of 68 (±10) years in REACH Western Europe and 70 (±10) years in REACH North America versus 60 (±10) years in UCC-SMART, and more patients with diabetes were enrolled; 34% in REACH Western Europe and 42% in REACH North America versus 17% in UCC-SMART. In UCC-SMART, more patients were current smokers; 31% versus 15% in REACH Western Europe and 13% in REACH North America. During a median follow-up time of 8.6 years (IQR 4.7-12.8) 2386 cardiovascular interventions occurred in the UCC-SMART cohort, and recurrent MACE was observed in 1671 patients. In participants from REACH Western Europe, during a median follow-up time of 1.75 years (IQR 1.50-2.25), 2272 interventions were performed, and 1776 recurrent MACE were observed. In REACH North America, during a median follow-up time of 1.75 years (IQR 1.50-1.83) 2194 interventions were registered, and 1988 participants were diagnosed with recurrent MACE. Outcome definitions and numbers are displayed in Table 2. Table 3 provides an overview of outcome numbers and incidence rates in strata of cardiovascular disease at baseline, and shows that outcome types vary for patients with CHD, CeVD, or PAD; for example, patients with PAD at baseline had more peripheral interventions and patients with CHD more cardiac interventions, and patients with CeVD had the fewest interventions overall.

Temporal validation of original SMART risk score in larger UCC-SMART dataset

Temporal validation of the existing SMART risk score in the larger UCC-SMART dataset provided a c-statistic of 0.69 (95%CI 0.68-0.71) (Appendix 2A). Calibration was good, with a slight overestimation in patients with a 10-year risk of >40% (Appendix 2B). Calibration in the larger UCC-SMART dataset in strata of cardiovascular disease at baseline was good (Appendix 3).

Development of the extended SMART risk score for MACE+

Transformations of continuous predictors, subdistribution hazard ratios and 95% confidence intervals of the model predictors are presented in Appendix 4. A shrinkage factor of 0.98 was observed and applied to shrink the model coefficients. The model formula that was used for the risk predictions is shown in Appendix 5.

External validation of the extended SMART risk score for MACE+

External validation of the risk model in the REACH cohorts, showed a c-statistic of 0.60 (95%CI 0.59-0.61) in REACH Western Europe, and 0.58 (95%CI 0.57-0.59) in REACH North America. Expected observed ratios were 0.96 and 0.82 in REACH Western Europe and REACH North America respectively. External calibration was good, as is shown in Figure 1. External validation in strata of cardiovascular disease at baseline in REACH Western Europe showed c-statistics of 0.60

(95%CI 0.59-0.61) for patients with CHD, 0.62 (95%CI 0.61-0.64) for CeVD, and 0.53 (95%CI 0.52-0.55) for PAD. Calibration was good for patients with CHD and CeVD, but poor calibration was observed for patients with PAD (Figure 2). In REACH North America, c-statistics were 0.57 (95%CI 0.56-0.59) in patients with CHD, 0.61 (95%CI 0.59-0.63) for CeVD, and 0.54 (95%CI 0.52-0.57) for PAD. Similarly, calibration was good in patients with CHD and CeVD, and poor calibration was observed in patients with PAD.

Table 1. Baseline characteristics for UCC-SMART, REACH W-Europe, and REACH N-America.

	UCC-SMART (N= 8,421)	**REACH W-Europe (N= 14,528)**	**REACH N-America (N= 19,495)**
Male, n (%)	6,214 (74%)	10,455 (72%)	12,080 (62%)
Age (years)*	60 ± 10	68 ± 10	70 ± 10
Current smoking, n (%)	2,573 (31%)	2,227 (15%)	2,548 (13%)
Medical history			
Cerebrovascular disease, n (%)	2,515 (30%)	4,536 (31%)	5,433 (28%)
Coronary artery disease, n (%)	5,155 (61%)	10,026 (69%)	15,719 (81%)
Peripheral artery disease, n (%)	1,486 (18%)	3,415 (24%)	2,370 (12%)
Abdominal aortic aneurysm, n (%)	711 (8%)	507 (4%)	795 (4%)
Years since first vascular event*	0 (0-4)	NA	NA
Diabetes Mellitus, n (%)	1,451 (17%)	4,888 (34%)	8,280 (42%)
Physical examination and laboratory measurements			
Body Mass Index (kg/m^2)*	27 ± 4	28 ± 4	29 ± 6
Systolic blood pressure (mmHg)*	139 ± 20	140 ± 19	132 ± 18
Diastolic blood pressure (mmHg)*	81 ± 11	80 ± 10	75 ± 11
Total cholesterol (mmol/L)*	4.7 (4.0 - 5.5)	5.1 ± 1.1	4.6 ± 1.0
HDL cholesterol (mmol/L)*	1.2 (1.0 - 1.4)	NA	NA
Hs-CRP (mg/L)*	2.0 (0.9 - 4.3)	NA	NA
Creatinine (µmol/L)*	92 ± 36	105 ± 84	114 ± 95
Medication			
Lipid lowering medication, n (%)	5,796 (69%)	10,331 (71%)	15,031 (77%)
Blood pressure lowering therapy, n (%)	6,316 (75%)	13,144 (90%)	18,237 (94%)
Anti-platelet therapy, n(%)	6,482 (77%)	9,669 (67%)	14,675 (75%)

* Data are displayed as mean (standard deviation) or median (quartiles)

UCC-SMART = Utrecht Cardiovascular Cohort-Second Manifestations of ARTerial disease

REACH = REduction of Atherothrombosis for Continued Health; W-Europe = Western Europe; N-America = North America

Table 2. Definitions and numbers of recurrent MACE and cardiovascular interventions in UCC-SMART, REACH W-Europe and REACH N-America

	UCC-SMART[14,24]	**N = 8,421**
Follow-up	Time to death or end of follow-up in years, median (IQR)	8.6 (4.7-12.8)
Combined endpoint	Recurrent major cardiovascular events (myocardial infarction, stroke, vascular death) and cardiovascular interventions	N=3,020 IR=5 / 100 PY
Non-fatal myocardial infarction	At least two of the following: 1. Chest pain; 2. ECG abnormalities; 3. CK elevation	N=595
Non-fatal stroke	Clinical features causing an increase of at least one grade on the modified Rankin scale and fresh infarct or hemorrhage on CT	N=424
Vascular death	Sudden death or death from stroke, myocardial infarction, congestive heart failure, rupture of abdominal aortic aneurysm, or from other cause, i.e. sepsis following stent placement.	N=962
Cardiac interventions	Percutaneous coronary intervention or coronary artery bypass surgery	N=1,634
Carotid artery interventions	Stent, angioplasty, (thrombo)endarterectomy, bypass surgery	N=246
Peripheral interventions (lower limbs)	Stent or graft (endovascular or open surgery), angioplasty, bypass surgery or urokinase treatment. Amputation lower limb due to arterial ischemia.	N=837

IR= Incidence rate (per 100 person-years). PY= person-years. W-Europe = Western Europe. N-America = North America. Number of events are given for specific outcomes, therefore the separate numbers do not exactly count up to the combined endpoint.

REACH[14,26]	**W-Europe** **N = 14,528**	**N-America** **N = 19,495**
Time to death or end of follow-up in years, median (IQR)	1.75 (1.50-2.25)	1.75 (1.50-1.83)
Recurrent major cardiovascular events (myocardial infarction, stroke, vascular death) and cardiovascular interventions	N=3,512 IR=13 / 100 PY	N=3,653 IR=12 / 100 PY
Self-reported, hospital documentation and confirmed by local physician	N=419	N=582
Based on information from neurologist or hospital report with diagnosis of stroke, ischemic or hemorrhagic	N=623	N=518
Sudden death or death from stroke, myocardial infarction, or other cardiovascular death: death following cardiovascular intervention, heart failure, visceral or limb infarction	N=878	N=1,069
Percutaneous coronary intervention (angioplasty ± stent), bypass surgery[37]	N=1,265	N=1,469
Stent, angioplasty, (thrombo)endarterectomy, bypass surgery[38]	N=278	N=328
Stent or graft (endovascular or open surgery), angioplasty, or bypass surgery. Amputation affecting lower limb due to arterial ischemia.[39]	N=988	N=610

Table 3. Recurrent MACE and cardiovascular interventions in UCC-SMART, REACH W-Europe and REACH N-America, in strata of cardiovascular disease at baseline

	Coronary heart disease	**Cerebro- vascular disease**	**Peripheral artery disease**
UCC-SMART	**N= 5,155**	**N= 2,515**	**N= 1,486**
Follow-up (years) to death or end of FU	*8.6 (4.6-12.6)*	*8.1 (4.3-12.3)*	*9.1 (5.0-13.6)*
Recurrent cardiovascular disease	1,012 (20%)	353 (14%)	406 (27%)
Carotid interventions			
Number (%)	113 (2%)	118 (5%)	70 (5%)
Incidence rate (/ 100 person-years)	0.25	0.57	0.52
Coronary interventions			
Number (%)	1,305 (25%)	279 (11%)	238 (16%)
Incidence rate (/ 100 person-years)	3.61	1.38	1.93
Peripheral interventions			
Number (%)	357 (7%)	165 (7%)	521 (35%)
Incidence rate (/ 100 person-years)	0.82	0.80	5.07
REACH Western Europe	**N= 10,026**	**N= 4,536**	**N= 3,415**
Follow-up (years) to death or end of FU	*1.8 (1.5-2.3)*	*1.8 (1.6-2.2)*	*1.8 (1.5-2.1)*
Recurrent cardiovascular disease	1,196 (12%)	707 (16%)	512 (15%)
Carotid interventions			
Number (%)	170 (2%)	114 (3%)	114 (3%)
Incidence rate (/ 100 person-years)	0.83	1.24	1.69
Coronary interventions			
Number (%)	1,080 (11%)	253 (6%)	286 (8%)
Incidence rate (/ 100 person-years)	5.49	2.79	4.29
Peripheral interventions			
Number (%)	542 (5%)	202 (5%)	676 (20%)
Incidence rate (/ 100 person-years)	2.70	2.22	10.76
REACH North America	**N= 15,719**	**N= 5,433**	**N= 2,370**
Follow-up (years) to death or end of FU	*1.8 (1.5-1.8)*	*1.8 (1.5-1.8)*	*1.8 (1.5-1.8)*
Recurrent cardiovascular disease	1,603 (10%)	718 (13%)	330 (14%)
Carotid interventions			
Number (%)	268 (2%)	98 (2%)	83 (4%)
Incidence rate (/ 100 person-years)	1.05	1.13	2.23
Coronary interventions			
Number (%)	1,351 (9%)	272 (5%)	151 (6%)
Incidence rate (/ 100 person-years)	5.49	3.18	4.11
Peripheral interventions			
Number (%)	465 (3%)	161 (3%)	309 (13%)
Incidence rate (/ 100 person-years)	1.84	1.87	8.75

All first events of a specific outcome are counted. Therefore carotid + coronary + peripheral interventions do not exactly count up to the number of all cardiovascular interventions

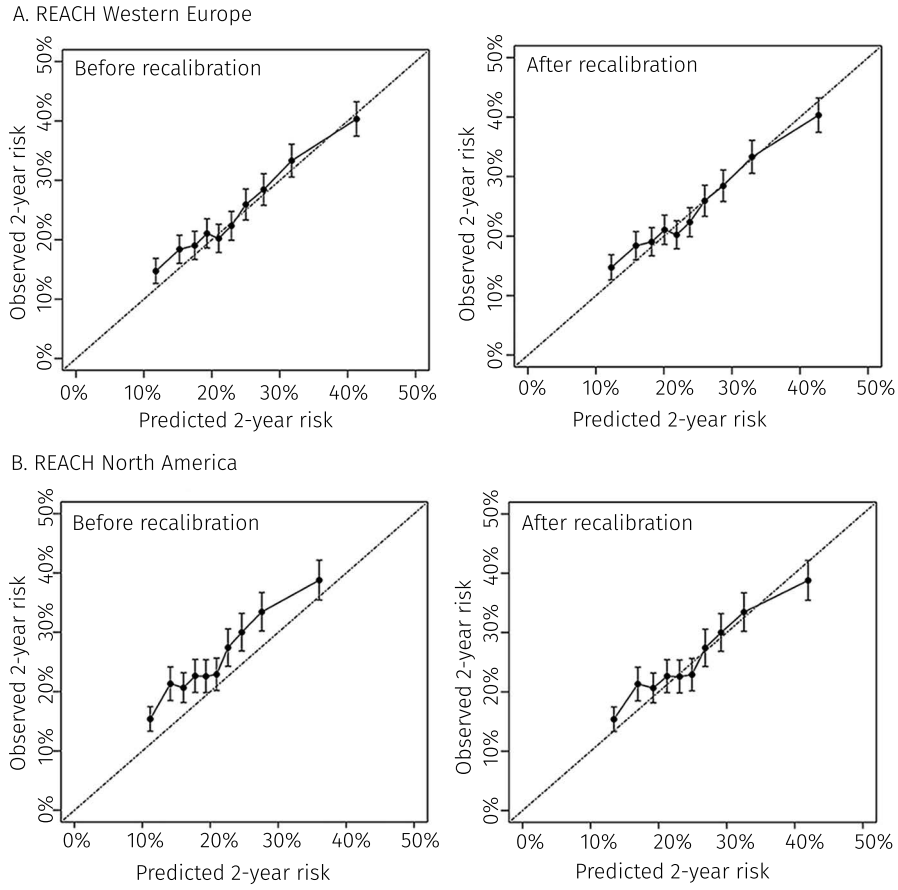

Figure 1. Plots of external calibration of the extended SMART risk score for MACE+ in REACH W-Europe and REACH N-America before and after recalibration

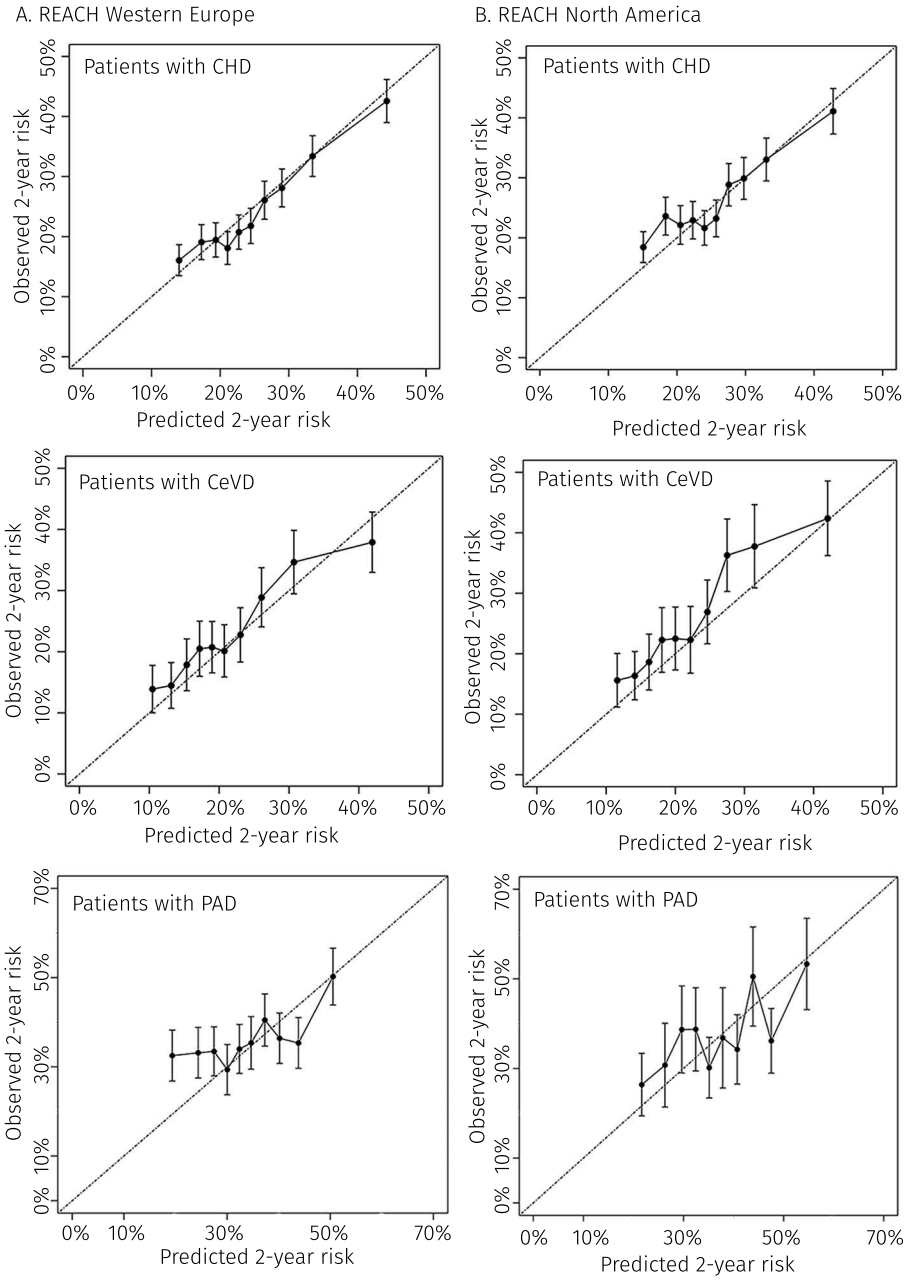

Figure 2. External calibration plots of the extended SMART risk score for MACE+ in strata of cardiovascular disease at baseline in REACH W-Europe and REACH N-America

Discussion

In patients with established cardiovascular disease, cardiovascular interventions are more common than major cardiovascular events. The 10-year risk of a combined outcome of recurrent cardiovascular events and cardiovascular interventions (MACE+) can be estimated in patients with established cerebrovascular and coronary heart disease by the currently developed prediction rule: the extended SMART risk score. Performance of the current residual cardiovascular risk model is inadequate in patients with established peripheral artery disease.

Validation of the current model showed good calibration and moderate discrimination with c-statistics of 0.60 and 0.58 in REACH Western Europe and North America respectively. In comparison, the original SMART risk score for estimating 10-year risk of major recurrent cardiovascular events in patients with established cardiovascular disease, showed c-statistics ranging from 0.62 to 0.66 upon external validation in seven datasets including the REACH registry.[14,40] Calibration of the SMART risk score in those 7 external datasets was good in patients with PAD and in general, even though miscalibration in REACH North America and slight overestimation of risk in patients with very high predicted risks (10-year risks of more than 40% and 2-year risk of more than 20%) was observed.[14,40] Discriminative power was slightly lower for the current model (extended SMART risk score) than the original SMART risk score, possibly due to the great diversity of the current outcome ranging from elective percutaneous interventions to vascular death. However, for assessment of prediction model performance, calibration is a more clinically relevant performance measure than discrimination with the c-statistic.[41] In short, it is more important to correctly estimate the risk in a given patient (calibration) then whether it discriminates between a high and low risk patient (discrimination and c-statistic).

In patients with peripheral artery disease, the model performed inadequately. Possible explanations for this inadequate performance concern both the outcome and the patient population. With regard to the outcome, in patients with peripheral artery disease, peripheral vascular interventions occurred more often, and these interventions are potentially challenging to predict. Predictors for a limb salvage operation due to critical limb ischemia might be very different from predictors for endovascular treatment of a restenosis. For example, salvage amputation is only performed when no other options are available or have already been tried and when the patient is not a candidate for extensive bypass surgery. Restenosis occurs quite frequently (18–40% within one year after stenting in the femoropopliteal segment[42,43]). The precise form, site and length of the endovascular intervention for PAD markedly influences restenosis risk, and thus earlier treatment influences the risk for new treatment, and these factors are not included in the model. As restenosis usually manifests between 3 and 6 months after initial intervention,[44] these patients will be regarded as high risk due to an early event, but might not necessarily have a very high risk factor profile. Although this could also be true

for coronary restenosis, restenosis is reported more often after peripheral interventions.[42,43,45] Additionally, in patients with a new diagnosis of claudication, indication for early peripheral vascular interventions depended on the treating physician.[46] It could be hypothesized that in patients with established peripheral artery disease, indication for peripheral (re-)intervention might also rely partly on clinician characteristics rather than patient factors. With regard to the patient population, patients with peripheral artery disease might have a less varied risk factor profile compared to patients with CHD or CeVD and consequently have fewer distinguishing factors for predicting higher or lower risk within this particular population.

Currently, the SMART risk score[17] and the SMART-REACH model[47] are the most used 10-year and lifetime residual risk prediction algorithms for patients with established CVD. The current model, the extended SMART risk score, estimating the risk of MACE+ will provide a valuable addition to those existing risk scores. Although the extended SMART risk score does not perform well in patients with PAD specifically, these patients often also have other types of cardiovascular disease, and are therefore seen by various specialists. The advantage of a general risk score applicable to all patients with any type of CVD is that it can be used by all types of specialists, and care for patients with established CVD will not become segregated. The combined outcome is highly diverse, but all outcomes could be regarded as clinically relevant from a patient's as well as from an economic perspective. Incidence rates of recurrent major cardiovascular events have declined[19] and the number of percutaneous cardiac revascularization procedures has risen quickly, with a more than 7 times increase in the United Kingdom from 1993 to 2013[48] and a more than double number in 2012 compared to a decade earlier in the Netherlands,[49] as a replacement for open surgery, and with expanding indications due to further developed technical options. It could be hypothesized that these trends will amplify over the next few years, and the risk of a combined endpoint of recurrent MACE and cardiovascular interventions (MACE+) might become a more fitting representation of an individual's true cardiovascular risk.

The current study had several strengths, including the large datasets enrolling patients with different types of established cardiovascular disease, and the long follow-up duration in the derivation dataset (UCC-SMART). Furthermore, due to adjustment for competing events accurate risk estimations of the event of interest are provided in a specific population that is also at risk of dying from other diseases, such as cancer. [50] By accounting for competing risks, overestimation of the event of interest is prevented.[51] However, limitations should be acknowledged and include the limited length of follow-up in the validation sets. Although the coefficients were the same for 10-year and 2-year risk predictions, the baseline hazard for 2-year risk predictions was separately derived from the derivation set and the assumption is made that the expected observed ratio for 2-year predictions is similar for 10-year risk predictions. Due to certain sampling methods for the REACH and UCC-SMART cohorts, it is possible that the absolute risk predictions are not applicable to all patients with established cardiovascular disease globally. There is no reason

to assume coefficients would be different, however, there might be variations in underlying baseline hazards. Lastly, indications for cardiovascular interventions or procedural information, such as location or length of the stent, were not available in the datasets.

In conclusion, the 10-year combined risk of recurrent cardiovascular events and cardiovascular interventions can be estimated in patients with established CHD or CeVD. However, cardiovascular interventions in patients with PAD cannot be predicted reliably.

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Appendices

Appendix 1. Definitions of baseline characteristics and outcomes of the UCC-SMART and REACH cohorts.

A. Definition of baseline characteristics

	UCC-SMART[1,2]	**REACH**[2,3]
Age	Years, reported by physician/patient	Years, reported by physician/patient
Sex	Male/female, reported by physician/patient	Male/female, reported by physician/patient
Current smoking	Current vs never/former, reported by patient	Current vs never/former, at least 5 cigarettes per day as a mean within the last month before entry into registry
History of diabetes mellitus	Either referral diagnosis, self-reported, or a known history of diabetes mellitus at the time of enrolment or a fasting blood glucose ≥7 mmol/L.	Any history of diabetes or current diabetes (diagnosed by at least 2 fasting blood glucose measures >7 mmol/L or >126 mg/dL), treated or not
Systolic blood pressure	mmHg. Measured directly after informed consent. Mean of two office blood pressure measurements.	mmHg. Measured in a seated position after at least 5 minutes of rest
Total cholesterol	mmol/L. Measured in fasting venous sample using commercial enzymatic dry chemistry kits (Johnson and Johnson)	Mg/dL. Transcribed from the clinical record, lipids were not measured in a standard manner in the registry participants
High density lipoprotein (HDL) cholesterol	mmol/L. Measured in fasting venous sample using commercial enzymatic dry chemistry kits (Johnson and Johnson)	Mg/dL. Transcribed from the clinical record, lipids were not measured in a standard manner in the registry participants
High sensitive C-reactive protein	Mg/L. Measured by immunonephelometry (Nephelometer Analyzer BN II, Dade-Behring). From 2013 determined in heparin plasma on an AU5811 routine chemistry analyzer (Beckman Coulter, Brea, California)	Not available
Estimated glomerular filtration rate (eGFR)	Estimated by CKD-EPI formula.[4] Creatinine measured in fasting venous sample using commercial enzymatic dry chemistry kits (Johnson and Johnson)	Estimated by CKD-EPI formula.[4] Serum creatinine measured at baseline

Time since first vascular event	Years. Reported by patient. When the patient's first cardiovascular event occurred in the preceding year, the duration of disease was rounded down to zero years.	Not available
History of peripheral artery disease	Symptomatic and documented obstruction of distal arteries of the leg of surgery of the leg (percutaneous transluminal angioplasty, bypass or amputation)	One or both of the following criteria: current intermittent claudication with ankle-brachial index of <0.9 or a history of intermittent claudication together with a previous and related intervention such as angioplasty, stenting, atherectomy, peripheral arterial bypass graft, or other vascular intervention, including amputation
History of coronary heart disease	Angina pectoris, myocardial infarction or coronary revascularization (coronary bypass surgery or coronary angioplasty)	Stable angina with documented coronary artery disease, history of unstable angina with documented coronary artery disease, history of percutaneous coronary intervention, history of coronary artery bypass graft surgery, or previous myocardial infarction
History of cerebrovascular disease	TIA, cerebral infarction, amaurosis fugax or retinal infarction, or a history of carotid surgery	Hospital or neurologist report with the diagnosis of TIA or ischemic stroke
History of abdominal aorta aneurysm	History or presence of aneurysm of abdominal aorta of >3cm or aortic surgery	History or presence of aneurysm of abdominal aorta

Appendix 1. Continued

B. Definition and numbers of recurrent major cardiovascular events

	UCC-SMART[1,2]	**REACH**[2,3]
Non-fatal myocardial infarction	At least two of the following: 1. Chest pain for at least 20 minutes, not disappearing after administration of nitrates; 2. ST-elevation >1 mm in two following leads or a left bundle branch block on the ECG; 3. CK elevation of at least two times the normal value of CK and a MB-fraction >5% of the total CK.	Self-report, hospital documentation and confirmed by local physician
Non-fatal stroke	Relevant clinical features which have caused an increase in handicap of at least one grade on the modified Rankin scale, accompanied by fresh infarct or hemorrhage on a repeat CT scan	Based on information from neurologist or hospital report with diagnosis of stroke, ischemic or hemorrhagic
Vascular death	Sudden death (unexpected cardiac death occurring within 1 hour after onset of symptoms, or within 24 hours given convincing circumstantial evidence) or death from stroke, myocardial infarction, congestive heart failure, rupture of abdominal aortic aneurysm, or from other cause, i.e. sepsis following stent placement.	Fatal stroke (within 28 days), fatal myocardial infarction (within 28 days), other cardiovascular death: other death of cardiac origin; pulmonary embolism; any sudden death including unobserved, and unexpected death (e.g. death while sleeping) unless proven otherwise by autopsy, death following a vascular operation, vascular procedure, or amputation; death attributed to heart failure; death following a visceral or limb infarction; and any other death that could not be definitely attributed to a nonvascular cause

Appendix 1. Continued

C. Definition and numbers of incident cardiovascular interventions*

	UCC-SMART[1,2]	**REACH**[2,3]
Heart	Percutaneous coronary intervention or coronary artery bypass surgery	Percutaneous coronary intervention (angioplasty ± stent), bypass surgery[5]
Carotid arteries	Stent, angioplasty, (thrombo) endarterectomy, bypass surgery	Stent, angioplasty, (thrombo) endarterectomy, bypass surgery[6]
Peripheral (lower limbs)	Stent or graft (endovascular or open surgery), angioplasty, bypass surgery or urokinase treatment. Amputation lower limb due to arterial ischemia. Other intervention due to ischemia (eg emergency laparotomy due to intestinal ischemia, nephrectomy due to atherosclerotic cause, kidney transplantation, acute abdominal aortic aneurysm or AAA intervention) NOT included**	Stent or graft (endovascular or open surgery), angioplasty, or bypass surgery. Amputation affecting lower limb due to arterial ischemia.[7]

* Patients who received a cardiovascular intervention in response to a cardiovascular disease event, are classified according to the cardiovascular disease event

** Other interventions due to ischemia are not documented in REACH and therefore not included in the endpoint

Hospitalization for transient ischemic attack (TIA) or unstable angina is not documented in UCC-SMART and therefore not included in the endpoint.

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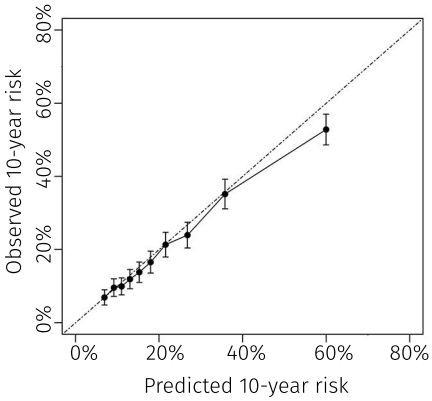
Appendix 2. Description of original derivation set and validation of SMART-risk score in current full UCC-SMART dataset

A. Description of datasets

	Dataset for original SMART risk score derivation[1]	**Current full dataset**
Number of patients	5788	8421
Follow-up (to first event)	4.7 (2.3-7.7)	8.6 (4.7-12.8)
Number of recurrent CVD events (defined as non-fatal MI, non-fatal ischemic stroke, vascular death)	788	1671
C-statistic SMART risk score	0.68 (0.64-0.71)	0.69 (0.68-0.71)



B. Calibration plot of original SMART-risk score in full current dataset

Reference

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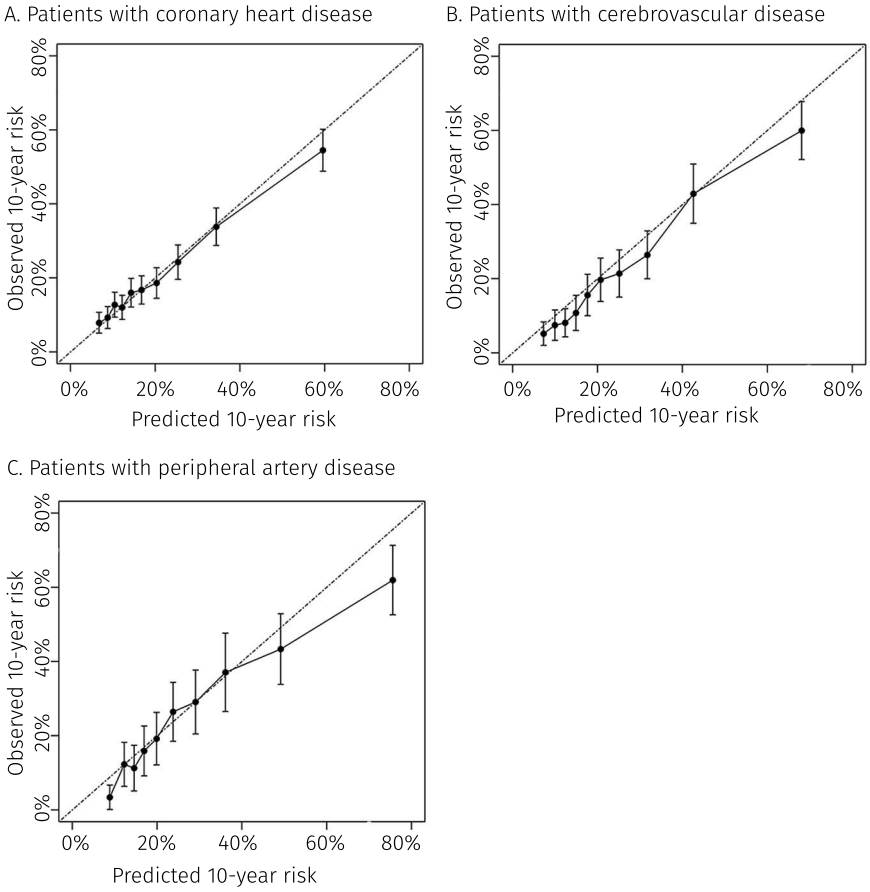

Appendix 3. Calibration plots of original SMART-risk score in current dataset in strata of cardiovascular disease at baseline

Appendix 4. Subdistribution hazard ratios with 95% confidence intervals of the model for cardiovascular disease and cardiovascular interventions

	Subdistribution hazard ratio	95% confidence interval
Age	1.012	1.007-1.016
Sex	1.245	1.136-1.465
Total cholesterol	1.529	1.224-1.910
Total cholesterol^2	0.969	0.949-0.990
Kidney function	0.982	0.971-0.994
Kidney function^2	1.000	1.000-1.000
Systolic blood pressure	1.004	1.002-1.006
HDL cholesterol	0.737	0.657-0.826
Log(C-reactive protein)	1.091	1.056-1.128
Years since first vascular event	1.050	1.035-1.066
Years since first vascular event^2	0.998	0.997-0.999
Current smoking	1.196	1.105-1.295
Diabetes mellitus	1.286	1.178-1.404
History of AAA	1.293	1.145-1.460
History of cerebrovascular disease	1.017	0.918-1.127
History of coronary heart disease	1.415	1.272-1.574
History of peripheral artery disease	1.753	1.583-1.941
Shrinkage factor 0.98		

Appendix 5. Computational formulas for 10-year risk of recurrent MACE and cardiovascular interventions

10-year risk of recurrent MACE and cardiovascular interventions (%) = (1 - 0.61785 ^ exp[A - 2.0869]) * 100%, where
A = 0.0116 x age in years + 0.2148 [if male] + 0.1754 [if current smoker] + 0.0037 x systolic blood pressure in mmHg + 0.2465 [if diabetic] + 0.3399 [if history of coronary artery disease] + 0.0167 [if history of cerebrovascular disease] + 0.2513 [if abdominal aortic aneurysm] + 0.5497 [if peripheral artery disease] + 0.0478 x years since first diagnosis of vascular disease - 0.0019 x (years since first diagnosis of vascular disease)^2 - 0.2989 x HDL-cholesterol in mmol/L + 0.4159 x total cholesterol in mmol/L - 0.0308 x (total cholesterol in mmol/L)^2 - 0.0177 x eGFR in mL/min/1.73m^2 + 0.0001 x (eGFR in mL/min/1.73m^2)^2 + 0.0854 x log(hs-CRP in mg/L) Coefficients after adjustment for the shrinkage factor (0.98) are presented.

Chapter 7

Prediction of lifetime and 10-year risk of cancer in individual patients with established cardiovascular disease

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Abstract

Background

Cardiovascular disease (CVD) and cancer share many common risk factors; patients with CVD also may be at risk of developing cancer

Objectives

The aim of this study was to derive and externally validate prediction models for the estimation of lifetime and 10-year risk for total, colorectal, and lung cancer in patients with established CVD.

Methods

Data from patients with established CVD from the UCC-SMART cohort (N=7,280) were used for model development, and from the CANTOS trial (N=9,322) for model validation. Predictors were selected based on previously published cancer risk scores, clinical availability, and presence in the derivation dataset. Fine and Gray competing risk-adjusted lifetime models were developed for the outcomes total, colorectal, and lung cancer.

Results

Selected predictors were age, sex, smoking, weight, height, alcohol use, antiplatelet use, diabetes, and C-reactive protein. External calibration for the 4-year risk of lung, colorectal, and total cancer was reasonable in our models, as was discrimination with C-statistics of 0.74, 0.64, and 0.63, respectively. Median predicted lifetime and 10-year risks in CANTOS were 26% (range 1%-52%) and 13% (range 1%-31%) for total cancer; 4% (range 0%-13%) and 2% (range 0%-6%) for colorectal cancer; and 5% (range 0%-37%) and 2% (range 0%-24%) for lung cancer.

Conclusions

Lifetime and 10-year risk of total, colorectal, and lung cancer can be estimated reasonably well in patients with established CVD with readily available clinical predictors. With additional study, these tools could be used in clinical practice to further aid in the emphasis of healthy lifestyle changes and to guide thresholds for targeted diagnostics and screening.

Introduction

Treatment for cardiovascular disease (CVD) has improved substantially over the past decades, with more patients surviving CVD and living long enough to develop other diseases such as cancer. Besides an increased risk of new cardiovascular events, patients with established CVD have a higher risk of cancer compared with the general population (standardized incidence ratio of 1.19; 95% CI 1.10-1.29 adjusted for age, sex and calendar year),[1] most likely due to several similar risk factors including obesity, smoking, and low-grade inflammation.[2,3] Furthermore, even though cardiovascular disease is still the leading cause of mortality worldwide among adults, in some higher and middle income countries cancer has become the predominant cause of death, partly due to improved prevention and treatment of CVD.[4]

Given one's absolute individual cancer risk varies, several risk prediction models have been developed to estimate the absolute risk for incident cancer of a specific type, notably lung cancer and breast cancer.[5-9] However, no prediction models are available for patients with established cardiovascular disease specifically. Furthermore, from a patient's perspective, risk of any cancer might be a more relevant metric, and no risk prediction models estimate total cancer risk. Furthermore, classic risk prediction models estimate prognosis in terms of absolute 5 or 10 year risk of cancer, and may not identify those patients who have a relatively low 5 or 10 year absolute risk, but a high cumulative lifetime risk.[10] Finally, traditional 10-year risk prediction scores often do not consider the competing risk of noncancer mortality, and are prone to several types of bias.[11] Especially in a population of patients with established CVD, the competing risk of noncancer mortality including cardiovascular death should be taken into account to prevent overestimation of cancer risk.

Estimating individualized probabilities could help in individual patients' and clinicians' understanding of cancer risk. As several modifiable risk factors are related to cancer[2], as well as to CVD, discussing these cancer risks with patients could potentially aid in emphasizing healthy lifestyle changes, such as smoking cessation or weight loss. The aim of the current study was to develop and externally validate prediction models to estimate the 10-year and lifetime risk for total, colorectal, and lung cancer in patients with established cardiovascular disease.

Methods

Study populations

Model development was conducted in the Utrecht Cardiovascular Cohort - Second Manifestations of ARTerial disease (UCC-SMART) study, an ongoing prospective cohort study, including 18-79 year-old patients referred to the University Medical Center Utrecht (UMCU) with clinically manifest vascular disease or atherosclerotic risk factors. The cohort was initiated in 1996 and is still recruiting patients annually. For the current study 7,280 patients aged 45 to 80 years with clinically manifest vascular disease and who gave permission for data requests to other medical authorities were included.

External model validation was performed in the CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study) (trial registration number: NCT01327846), a double-blind, placebo-controlled, randomized clinical trial, that included 10,061 participants with a myocardial infarction at least one month prior to study entry and elevated C-reactive protein (CRP) concentration (≥2 mg/L). Eligible patients were randomized to receive either placebo, or canakinumab at a dose of 50mg, 150mg, or 300mg.[12] For the current study 9,322 patients were included, after exclusion of patients younger than 45 or older than 80 years of age. Detailed descriptions of the UCC-SMART cohort and the CANTOS trial have been published elsewhere.[12-14] The studies were approved by institutional review boards and all participants provided written informed consent.

Outcomes

During follow-up, participants enrolled in the UCC-SMART cohort received biannual questionnaires, gathering information on occurrence of recurrent CVD, bleeding events, incident diabetes mellitus, and end stage renal disease. Additional information was collected from hospital or general practitioner's data. An endpoint committee of three physicians adjudicated all clinical events independently and conflicting classifications were resolved in consensus. For data on cancer incidence, the UCC-SMART database was linked to the Dutch National Cancer Registry (INKL), a national registry receiving notifications of all new cancer diagnoses in the Netherlands through the Nationwide Network and Registry of Histopathology and Cytopathology (PALGA), and hospital discharge diagnoses.

Participants in the CANTOS trial were followed up for incident cardiovascular disease as well as cancer diagnoses. Even though the primary endpoint of the trial was CVD incidence, patients' records were investigated for cancers reported during the follow-up, as prespecified in the trial safety monitoring plan. Incident cancer reports were classified by an endpoint committee of oncologists, blinded for treatment allocation.[15] An overview of cancer diagnoses during follow-up for both study populations is provided in Appendix 1 and Appendix 2. For the current study, total cancer was defined as any invasive neoplasm, excluding non-melanoma skin cancer. As

lung and colorectal cancer are the most common (not sex specific) cancers worldwide[16], these were chosen as separate outcomes. For the endpoint of total cancer, only first diagnoses of cancer were counted. For lung and colorectal cancer, the first diagnosis of that particular cancer type was included, possibly being the second or third primary diagnosis of cancer for a certain patient during follow-up.

Data preparation and predictor selection

Missing data (per variable ≤1.1% for UCC-SMART and ≤0.2% for CANTOS) were singly imputed by weighted probability matching using multivariate regression with baseline as well as outcome data. Complete case analysis yielded similar model coefficients. Continuous variables were truncated at the 1st and 99th percentile to limit the effect of outliers on the model coefficients (i.e., leverage).[17] To prevent overfitting, predictors were preselected based on presence in previously published risk prediction models of multiple cancer types. Antiplatelet use (aspirin, P2Y12-ADP receptor antagonist, or other, such as dipyridamole) was added as a predictor, due to its inclusion in multiple previously published prediction models for colorectal cancer and due to the common use of antiplatelet therapy in patients with CVD. Furthermore, it was required that the variables were readily clinically available, as well as present in the derivation set. This led to the following predictors: age, sex, smoking status, weight, height, alcohol use, use of antiplatelet medication, and diabetes mellitus (Appendix 3 details an overview of predictor selection). In addition, CRP was added as a predictor after a literature search for predictors of cancer was performed.[15,18-20] Definitions of the predictors in the UCC-SMART cohort and CANTOS trial are provided in Appendix 4.

Development of a prediction model for total cancer, colorectal cancer, and lung cancer

Methods have been described in detail previously.[10,11] Three separate complementary Fine and Gray competing risk-adjusted subdistribution hazard functions[21,22] with left truncation and right censoring were developed in the UCC-SMART cohort for 10-year and lifetime risk predictions of 1) total cancer, 2) colorectal cancer, and 3) lung cancer, and for their competing mortality; 1) non-cancer death, 2) non-colorectal cancer death, and 3) non-lung cancer death. As the endpoints colorectal and lung cancer included potential second or third primary diagnoses of cancer for a particular patient, the competing risks for these outcomes did not include other cancer types. The models were developed with left truncation: age rather than follow-up time was used as the underlying time scale. This way, patients contributed personyears between age at study entry and age at study exit, resulting in overlapping observations that allow for lifetime predictions across the range of baseline ages. Because a limited number of patients and events in certain age groups led to instability of predictions, the age range at baseline was restricted to 45 to 80 years.

The proportional hazards assumption was assessed visually by plotting scaled Schoenfeld residuals against time, and interactions with age (underlying time scale) were added to the model when a violation was observed. Log and quadratic associations between continuous predictors and the outcome variable were assessed by comparing model fit based on Akaike's Information Criterion (AIC),[17] and transformations were applied when appropriate to improve robustness of the model. AICs of models with and without addition of CRP as a predictor were compared to assess differences in model fit. Coefficients of the predictors were adjusted to account for optimism using a shrinkage factor acquired by bootstrapping with a 1,000 bootstrap samples.

Individual cancer risk predictions

Individual 10-year and lifetime risk of total, colorectal, and lung cancer, as well as life expectancy without cancer were estimated using the respective models. These predictions can be derived from an individual lifetable with one year time intervals.[23] First, starting at the baseline age for each patient, the risk of the event of interest (a_t) and the risk of the competing event (b_t) was calculated for each following life-year. Next, for each subsequent age year the probability of being healthy and alive at the start of that time interval (age year) (e_t+1) was calculated by multiplying the survival probability (e_t) by the event-free survival probability during that year (1 - a_t - b_t). These steps were repeated from the age at baseline of an individual patient to the maximum age of 90 years, and together these predictions form an individual lifetable.[10,24] The cancer-free life expectancy was determined as the age where the median estimated cancer-free survival curve is 50%. For 10-year and lifetime risk of cancer, the cumulative cause-specific risks were truncated at 10 years after the age at baseline, and at the age of 90 respectively.

Internal and external validation of validation of the models

Internal validation of the total cancer, colorectal cancer, and lung cancer models was performed at 10-years of follow-up in the UCC-SMART data. External validation of the total, colorectal, and lung cancer models was evaluated in outcome data from the CANTOS trial at 4 years of follow-up (approximation of the median follow-up time in the CANTOS trial) by implementing the 4-year baseline hazard from the derivation dataset (UCC-SMART). To adjust for treatment effects of canakinumab, hazard ratios of treatment effects of canakinumab on cancer outcomes and their competing mortality were determined and added to the respective models. Discrimination was assessed using Harrell's c-statistic for survival data, and goodness of fit was assessed by calibration plots of the predicted versus observed risks. For the calibration plot, patients were divided into equal groups of increasing predicted risk. Based on the number of events, patients were divided into 10 equal groups for the total cancer model, and patients were divided into 6 equal groups for the colorectal and lung cancer models. Observed risks were estimated in these groups by using a cumulative incidence function, accounting for competing risks. Recalibration

was performed based on the expected to observed ratio. Predicted risks in the CANTOS trial were estimated after recalibration. The Brier score was calculated for 4-year predictions in CANTOS, with confidence intervals based on the percentile method with 1,000 bootstrap samples with replacement.

For comparison, simple models for total, colorectal, and lung cancer with sex and smoking status as the only predictors and with age as underlying time scale were developed in the UCC-SMART study and externally validated in the CANTOS study population by the same methodology.

All analyses were performed in R statistical software, version 3.5.1 for model development, and 3.6.0. for external validation analyses (packages Hmisc, rms, cmprsk, car). To facilitate the use of this model in clinical practice, an online calculator will be developed.

Results

Baseline characteristics of the UCC-SMART and CANTOS study populations are shown in Table 1. During a median follow-up time of 8.1 years (interquartile range (IQR) 4.5-12.1 years) a total number of 1,143 (first) cancers were diagnosed in patients enrolled in the UCC-SMART cohort. Lung cancer occurred in 258 patients and colorectal cancer in 180 patients. Incidence rates for total cancer and non-cancer mortality (competing event) were 1.97 (95%CI 1.85-2.08) and 1.91 (95%CI 1.80-2.02) per 100 person-years, respectively. Median follow-up time of the CANTOS trial was 3.8 years (IQR 3.2-4.5), during which a total number of 509 incident cancers were diagnosed, 123 lung cancers, and 72 colorectal cancers. Incidence rates of total cancer and non-cancer mortality were 1.48 (95%CI 1.35-1.61) and 2.21 (95%CI 2.05-2.37) respectively. An overview of incidence rates is shown in Appendix 5.

Development of lifetime risk prediction models for colorectal, lung, and total cancer in UCC-SMART

Results of model development are shown in Appendix 6 to 9. Transformations of continuous predictors, and interactions with age for continuous as well as categorical predictors are shown in Appendix 6. Age-specific baseline survival is shown in Appendix 7. Subdistribution hazard ratios and shrinkage factors are shown in Appendix 8, and model formulas of the total cancer, colorectal cancer, and lung cancer models are shown in Appendix 9. The AIC was lower for total cancer, colorectal cancer, and lung cancer models with CRP compared with the same model without CRP.

Internal and external validation of total, colorectal, and lung cancer models

Internal validation showed good agreement between the predicted and observed 10-year risk for total, colorectal, and lung cancer (Appendix 10) and c-statistics were 0.61 (95%CI 0.59-0.63), 0.61 (95%CI 0.57-0.66), and 0.74 (95%CI 0.70-0.77) respectively in the UCC-SMART study population.

External calibration plots in figure 1A-C show reasonable agreement between the predicted and observed 4-year risk for total, colorectal, and lung cancer in the CANTOS study population. The expected observed ratios of the event of interest and competing event were 1.06 and 0.99 for the total cancer model, 1.16 and 0.85 for the colorectal cancer model, and 0.60 and 0.99 for the lung cancer model, accounting for difference in baseline risk. Assessment of discrimination provided a c-statistic of 0.63 (95%CI 0.61-0.66) for the total cancer model, 0.64 (95%CI 0.58-0.70) for the colorectal cancer model, and 0.74 (95%CI 0.70-0.78) for the lung cancer model in the CANTOS data. Appendix 11-13 show calibration plots and c-statistics for the competing risks and cancer-free survival of the total, colorectal, and lung cancer models. The Brier score for 4-year predictions of total, colorectal, and lung cancer was 0.052; 95% CI: 0.048 to 0.057, 0.008; 95% CI: 0.006 to 0.010, and 0.013; 95% CI 0.010 to 0.016, respectively.

Table 1. Baseline characteristics of UCC-SMART and CANTOS study populations

	UCC-SMART (N=7,280)	**CANTOS** (N=9,322)
Male, n (%)	5470 (75%)	6869 (74%)
Age (years)*	62 ± 9	62 ± 8
Former smoking, n (%)	3582 (49%)	4437 (48%)
Current smoking, n (%)	2146 (29%)	2197 (24%)
Alcohol consumption >0 and <10 units per week, n(%)	3850 (53%)	1654 (18%)
Alcohol consumption >10 units per week, n(%)	2173 (30%)	1124 (12%)
Medical history		
Cerebrovascular disease, n (%)	2128 (29%)	712 (8%)
Coronary heart disease, n (%)	4530 (62%)	9322 (100%)
Peripheral vascular disease, n (%)	1300 (18%)	844 (9%)
Diabetes Mellitus, n (%)	1321 (18%)	3829 (41%)
Physical examination and laboratory measurements		
Body Mass Index (kg/m2)*	27 ± 4	31 ± 6
Systolic blood pressure (mmHg)*	140 ± 20	130 ± 16
Diastolic blood pressure (mmHg)*	81 ± 11	78 ± 9
LDL cholesterol (mmol/L)*	2.7 (2.1-3.5)	2.1 (1.7 - 2.8)
C-reactive protein (mg/L)*	2.0 (0.9 - 4.4)	4.2 (2.8 - 7.1)
Creatinine (µmol/L)*	91 ± 23	86 ± 29
Medication		
Lipid lowering medication, n (%)	5038 (69%)	8711 (93%)
Blood pressure lowering medication, n (%)	5549 (76%)	7591 (81%)
Anti-platelet therapy, n(%)	5652 (78%)	8488 (91%)
Anti-coagulants, n (%)	816 (11%)	718 (8%)

* Data are displayed as mean (standard deviation) or median (25th and 75th percentile)
UCC-SMART = Utrecht Cardiovascular Cohort-Second Manifestations of ARTerial disease; CANTOS = Canakinumab Anti-Inflammatory Thrombosis Outcomes Study; LDL = Low density lipoprotein.

Compared with a simple model with sex and smoking status as only predictors and with age as underlying time scale, the full model had a better fit according to the likelihood ratio test for total and lung cancer (p-values 0.005 and <0.001 respectively). For the colorectal cancer model, the full model did not improve model fit (p-value 0.174). Although the c-statistics of the simple models in CANTOS were similar or even slightly higher; 0.65; 95%CI 0.62-0.67 for total cancer, 0.65; 95%CI 0.62-0.66 for colorectal cancer, and 0.74; 95%CI 0.70-0.79 for lung cancer, and although calibration was similar for colorectal and lung cancers, calibration was worse for total cancer and for the competing risks (Appendix 14). As calibration is a more clinically relevant

performance measure for risk prediction accuracy than the c-statistic,[25] the full model for total cancer was considered superior. As all predictors are needed for estimations of total cancer risk, the advantage of a simple model with a limited number of predictors was no longer relevant, and full models were used for risk predictions of total, colorectal, and lung cancer.

Predicted 10-year and lifetime risk of cancer

Median predicted absolute 10-year risks were 13% (range 1%-31%) for total cancer, 2% (range 0%-6%) for colorectal cancer, and 2% (range 0%%-24%) for lung cancer in the CANTOS study population. In the UCC-SMART study population, predicted 10-year risks were 16% (range 2%-33%) for total cancer, 2% (range 0%-5%) for colorectal cancer, and 2% (range 0%-20%) for lung cancer. Median predicted absolute lifetime risks were 26% (range 1%-52%) for total cancer, 4% (range 0%-13%) for colorectal cancer, and 5% (range 0%-37%) for lung cancer in the CANTOS study population. In the UCC-SMART study population, median predicted absolute lifetime risks were 35% (range 2%-59%) for total cancer, 5% (range 0%-11%) for colorectal cancer, and 7% (range 0%-32%) for lung cancer. Median predicted 10-year and lifetime risks per age group with a 5-year interval for the UCC-SMART and CANTOS study populations are provided in Appendix 15. The distribution of lifetime risks for total, colorectal and lung cancer for UCC-SMART and CANTOS study populations is shown in Figure 2A-C.

As an example, for a 50-year old male with average values for all other predictors, his predicted lifetime risk of total cancer is 48% if he is a current smoker, 45% if he is a former smoker, and 35% if he has never smoked. The predicted lifetime risks of colorectal cancer for this 50-year old male are 6% (current smoker), 7% (former smoker), and 6% (never smoker). This 50-year old male has a predicted lifetime risk of lung cancer of 18% if he is a smoker, 10% if he is a former smoker, and 4% if he is a never smoker.

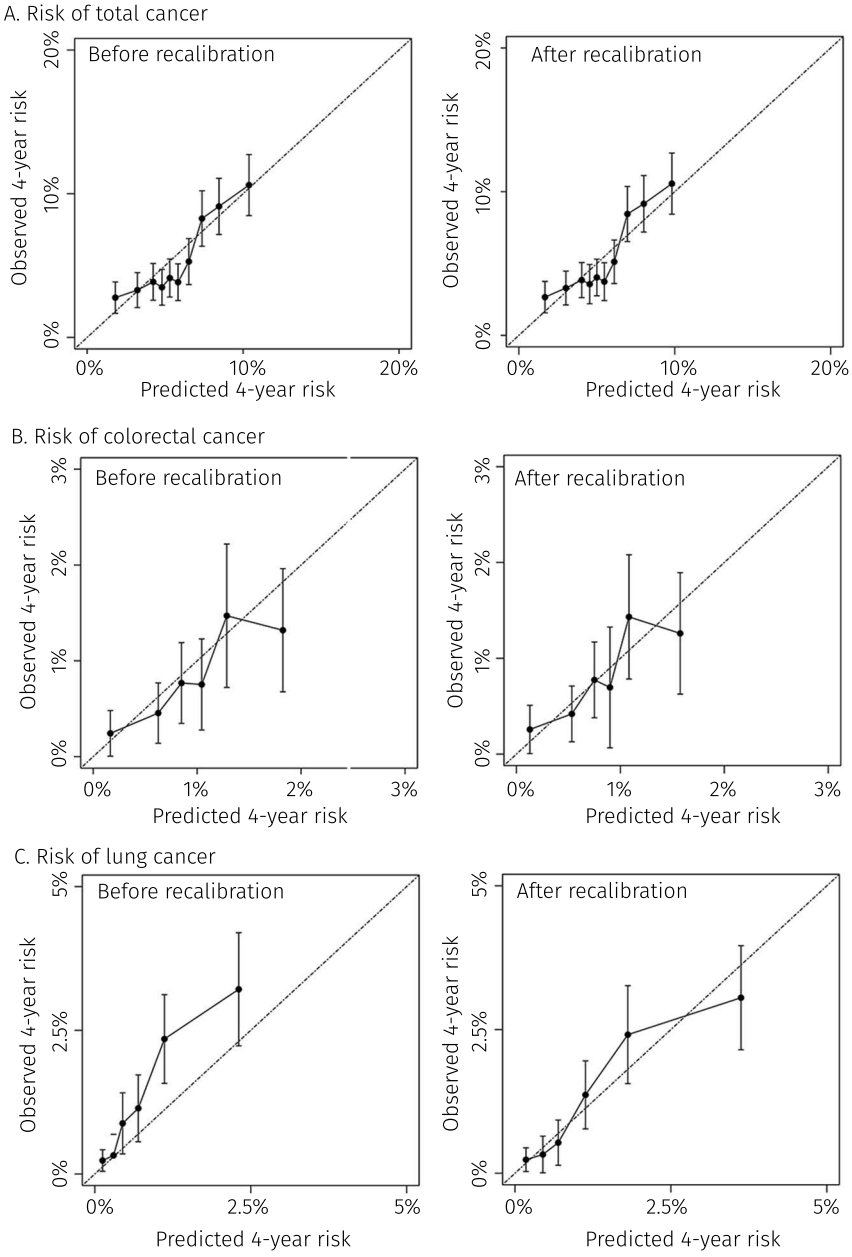

Figure 1. External calibration in the CANTOS trial population of cancer models before and after recalibration Calibration plots are shown of the predicted versus observed 4-year risk of total, colorectal, and lung cancer in the CANTOS study population, before and after recalibration. The study population is divided into quantiles based on the predicted risk, and ordered according to increasing predicted risk. The diagonal dotted line represents perfect calibration.

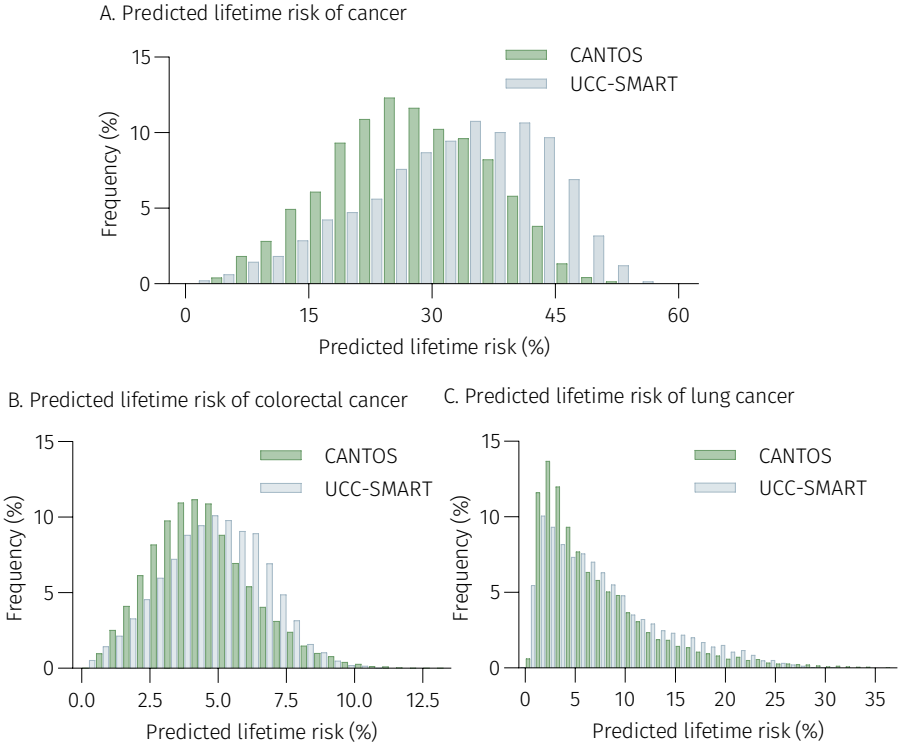

Figure 2. Distribution of predicted lifetime risk for total cancer, colorectal cancer, and lung cancer
Histograms show the distribution of the predicted lifetime risk for total, colorectal, and lung cancer in the UCC-SMART and CANTOS study populations. The predicted lifetime risk is the predicted risk of incident cancer up to the age of 90. Presented predicted risks are estimated by the full models for total, colorectal, and lung cancer.

Discussion

The present study demonstrates that lifetime and 10-year risk of total, colorectal, and lung cancer can be estimated reasonably well in individual patients with established CVD. Although discrimination was moderate with c-statistics of 0.63 to 0.74, calibration of the total, colorectal, and lung cancer models was reasonable. Given the wide distribution of predicted lifetime risks for total cancer and lung cancer (Figure 2A-C), these models can enable the identification of patients at the highest risk for cancer. Innovative and notable aspects of our work include the applicability to patients with established CVD specifically; the relative ease of use with readily clinically available predictors; the prediction of the combined endpoint total cancer; the external validation; and the estimation of lifetime risks with adjustment for competing risks.

Several risk prediction models with clinical predictors have previously been published for specific types of cancer, including lung,[5,6] colorectal,[6,26-29] and breast[6-9] cancer. None of these models were developed for patients with established CVD specifically, even though these patients are at higher risk for total and lung cancer compared to the general population, with standardized incidence ratios of 1.19 (95% CI 1.10-1.29) for total cancer and 1.56 (95% CI 1.31-1.83) for lung cancer (for colorectal cancer 1.08 (95% CI 0.86-1.34)),[1] due to similar risk factors for CVD and cancer.[2] Furthermore, the endpoint total cancer will have a different distribution of cancer types in patients with established CVD,[1] and patients with established CVD are at higher risk for the competing risk, i.e. dying from CVD, compared to the general population,[30] emphasizing the need for a prediction model in patients with established CVD specifically. It has even been hypothesized that CVD itself influences cancer development, for example through cardiac excreted factors in heart failure,[31,32] potentially leading to a higher baseline risk independent of traditional risk factors. Even though cancer is a very heterogeneous disease and prognoses are divergent for the various cancer types, from a patient's perspective risk of any cancer will be relevant, with respect to the potential mortality and morbidity associated with the malignancy, frequent hospital visits, demanding treatments,[33] and psychological distress.[34,35] Furthermore, in patients with CVD specific cancer types are more common, for example cancer of the respiratory tract,[1] leading to restricted variation in cancer types.

Our cancer prediction models performed reasonably well, and calibration plots before and after recalibration were similar. Only lung cancer risk was slightly underestimated in the CANTOS trial population before recalibration, probably due to variations in smoking habits, or genetic factors causing a higher baseline risk. The higher discriminative power of the lung cancer model (c-statistic 0.74) compared with the total and colorectal cancer models (c-statistics 0.63 and 0.64, respectively), is possibly due to the strong relation between the predictor smoking status and lung cancer. For the prediction of lung and colorectal cancer a simple model with just age, sex, and smoking status could be sufficient, however, for total cancer and the competing risks

the full model was necessary to achieve accurate predictions. For lung cancer, even though the calibration plot showed a 4-year risk of ±3% in the highest risk group, the model allowed for a widespread lifetime risk distribution, assigning lifetime risks up to 37% to a small proportion of patients. As young patients generally have a low 10-year risk of cancer, despite high risk factor levels, lifetime risk predictions might provide more accurate estimations of their 'true' risk. The lifetime risk of cancer estimated by the total cancer model ranges from 1% to 52%, enabling identification of patients at the highest risk. Median predicted risks for total cancer were higher in the UCC-SMART study population, corresponding with a higher observed incidence rate for total cancer (1.97 versus 1.48 per 100 person-years), most likely due to more current smokers in UCC-SMART compared to CANTOS (29% versus 24%). The distribution of colorectal cancer risk predictions is slightly limited, possibly partly due to absence of family history of colorectal cancer as a predictor in the model, and this model might be less appropriate for selecting patients at very high risk for colorectal cancer.

C-reactive protein was included in the risk prediction models based on previous observational research showing a relation between CRP and incident (lung) cancer,[3,19,20] and based on results from the CANTOS trial demonstrating that lowering inflammation with an IL-1β inhibitor lowered the incidence of lung cancer and lung cancer mortality.[15] Implementing CRP as a marker of low-grade inflammation in risk scores for determining cancer risk could lead to more accurate predictions. In current models for total, colorectal, and lung cancer CRP improved model fit based on the AIC. Previous research showed that CRP improved discrimination in a prediction model for lung cancer in the general population, but only for diagnoses within the first two years after measuring CRP.[19] In the current models for total and lung cancer, an interaction with age leads to a higher coefficient of CRP in increasing age, potentially representing a higher predictive value of CRP closer to cancer diagnosis.

There are multiple potential applications of this work, which each require further study. Personalized risk assessment is considered informative and motivating by patients,[36] and effective risk communication can lead to changes in behavior.[37] Although observed effects of personalized risk communication on healthy behavior changes were small and evidence is inconsistent,[38] effects are dependent on representation of risk information.[37] Lifetime risk predictions for cancer, especially in patients at a younger age, could potentially aid in discussions on the importance of healthy lifestyle habits and might increase patients' efforts to improve lifestyle, including smoking cessation. Future prospective studies are needed to evaluate lifestyle improvements and clinical outcomes in patients at high risk for cancer identified by these current models. Moreover, we hypothesize that these models could be used to further inform screening. Results from a recent lung cancer screening trial (NELSON [Nederlands–Leuvens Longkanker Screenings Onderzoek] trial) showed that screening for lung cancer could reduce lung cancer mortality in men (cumulative rate ratio for death from lung

cancer at 10 years of 0.76; 95% CI: 0.61 to 0.94).[39] The NELSON trial included 50- to 74-year-old current or former smokers who had smoked more than 15 cigarettes a day for more than 25 years or more than 10 cigarettes a day for more than 30 years, and showed a 10-year risk for lung cancer of approximately 6% in the screening group (incidence rate of 5.58 cases per 1000 person-years).[39] Similarly, it could be hypothesized that patients with stable CVD with a high 10-year predicted risk of lung cancer may benefit from screening computed tomography imaging of the chest. A predicted 10-year lung cancer risk of 6% (close to the 90th percentile in CANTOS) that corresponds to the observed risk in the NELSON study, could potentially be used as one threshold. In addition, application of the predicted lung cancer risk could be used to inform thresholds for targeted diagnostics in patients with early symptoms and high predicted 10-year risks, potentially leading to earlier detection and treatment of cancer.

Strengths of the present study include the large study populations for development and external validation of the cancer risk prediction models. Another important strength is the competing risk adjusted analyses, preventing overestimation of the event of interest, especially in a population of patients with established cardiovascular disease. Furthermore, by using age as the underlying time scale in the models, predictions are not limited by follow-up time in the derivation cohort and lifetime predictions are enabled. Last, the prediction model will be available in the online supplemental file. Limitations, however, should be considered. These include the limited number of lung cancer and colorectal cancer in the development and validation study populations. Furthermore, external validation in the CANTOS trial could be performed only up to 4 years, due to limited length of follow-up, although internal validation of 10-year predictions in UCC-SMART showed good calibration. Previous studies have shown that lifetime predictions based on the current methodology provide adequate estimates for up to at least 17 years,[10] and the advantage of CANTOS is the large number of patients with CVD and detailed information on incident cancer. C-statistics for the total cancer, colorectal cancer, and lung cancer models are moderate (0.62-0.74), comparable to previous cancer risk predictions models[5,7,26] and recurrent CVD risk prediction models in patients with established vascular disease.[24,40,41] However, evaluation of discrimination with the c-statistic is not optimal in assessing performance of risk prediction models. Calibration is a more clinically relevant performance measure for risk prediction accuracy.[25] Calibration of the total, colorectal, and lung cancer predictions models in the CANTOS trial population were all reasonable. Although patients were included in stable phase after a qualifying cardiovascular event, patients potentially changed lifestyle habits such as smoking during follow-up, and the single baseline measurement might not reflect time varying covariates. Last, several potentially important predictors, including level of education, socioeconomic status, race, and family history of cancer were unavailable in the derivation cohort and could not be included in the prediction models, possibly limiting model performance.

To conclude, lifetime and 10-year risk of total cancer, colorectal cancer, and lung cancer can be estimated reasonably well with easy clinically available predictors in patients with established CVD. The wide distribution of predicted lifetime risks for total and lung cancer enables identification of patients at the highest risk for cancer. With additional study, the lifetime total and lung cancer models could be used in clinical practice to further promote healthy lifestyle changes, and application of these models, particularly the10-year lung cancer risk model, could potentially lower thresholds for targeted diagnostics and screening.

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Appendices

Appendix 1. Cancer diagnoses according to ICD-10 classification in UCC-SMART cohort

Topography	Number	ICD-10 code
Colorectum	177	C18-C20
Lung, bronchus	226	C34
Breast	70	C50
Prostate	188	C61
Kidney, renal pelvis, ureter	52	C64-C66
Bladder, or unspecified parts of urinary organs	57	C67-C68
Hodgkin's disease	1	C81
Non-Hodgkin's lymphoma	30	C82-C85
Multiple myeloma	19	C88, C90
Leukemia	32	C91-C96
Melanoma of skin	52	C43
Lip, oral cavity, pharynx	31	C00-C14
Esophagus	36	C15
Stomach	38	C16
Small intestine	6	C17
Liver and bile ducts, gallbladder	21	C22-C24, C26.9
Pancreas	34	C25
Nasal cavity, middle ear, accessory sinuses, larynx, trachea	26	C30-C33
Bone and articular cartilage of limb	1	C40-C41
Mesothelial and soft tissue	20	C45-C49
Vulva or vagina	5	C51-C52
Cervix uteri or corpus uteri	14	C53-C54
Ovarium	5	C56-C57
Penis or testis	5	C60,C62-C63
Eye, brain, and other parts of central nervous system	8	C69-C72
Thyroid gland	3	C73
Ill-defined, secondary and unspecified sites	22	C76-C80

Appendix 2. Cancer diagnoses according to cancer endpoint committee in CANTOS trial

Topography	Number
Colorectum	72
Lung, bronchus	123
Breast	24
Genitourinary	102
Hematologic	27
Skin (incl. melanoma, basal cell, squamous cell)	156
Kidney	28
Liver	20
Central nervous system	11
Endocrine (incl. thyroid and adrenal)	10
Sarcoma	5
Other	24

Appendix 3. Predictor selection based on previously published cancer risk prediction models

	5 Most common cancers according to WHO[1]	
	Lung	**Colon**
Number of prediction models (reference)	9[2] + 1[3]	9[4] + 1[5] + 1[6] + 1[3] +1 [7]
Predictors (number of occurrences in prediction models)		
Smoking	Smoking status (N=5), Smoking intensity (e.g. pack-years) (N=7), Duration of smoking (N=8), Quit years of smoking (N=4), Age started smoking (N=1)	Smoking status (Men N= 4) (Women N= 4) (Both N=5)
Body mass index	BMI (N=3)	BMI (Men N= 5) (Women N= 3) (Both N=5)
Alcohol use		Alcohol (Men N= 4) (Women N= 3) (Both N=7)
Diabetes/glucose		Diabetes (Men N=3) (Women N=3) (Both N=1)
Height		Height (Both N=1)
Other		Aspirin/NSAID use (Men N= 2) (Women N= 2) (Both N=2)
Cancer in medical history*	Cancer in medical history (N=2)	Blood cancer (Men N= 1), Lung cancer (Men N= 1), Oral cancer (Men N= 1), Cancer in medical history (Men N=1) (Women N= 2)

	Prostate	Breast	Gastric
	1[3] + 1[8] + 1[9]	17[10] + 2[11] + 1[12] + 1[3]	1[3] + 1[13] + 1[14]
	Smoking status (N=2)	Smoking status (N=1)	Smoking status (Men N=1) (Women N=1) (Both N=2)
	BMI (N=2)	BMI (N=11)	BMI (Men N=1) (Women N=1)
	Alcohol consumption (N=1)	Alcohol (N=7)	Alcohol (Men N=1) (Women N=1)
	Diabetes/glucose (N=2)		Diabetes/HbA1c (Men N=1) (Women N=1) (Both N=1)
		Height (N=5)	
		Cancer in medical history (N=1)	Cancer in medical history (Men N=1) (Women N=1)

Appendix 3. Predictor selection based on previously published cancer risk prediction models

	5 Most common cancers according to WHO[1]	
	Lung	**Colon**
Race/ethnicity*	Race (N=4)	Ethnicity (Men N= 4) (Women N= 4)
Education/social economic status*	Education (N=3), Deprivation index (N=1)	Education (Men N=1) (Women N=1), Deprivation (Men N= 2)
Family history of cancer*	Family history of lung cancer (N=7)	Family history of cancer (Men N= 5) (Women N= 4) (Both N=3)
Dietary factors*		(Red) meat intake (Men N= 1) (Women N= 1) (Both N=2), Vegetable intake (Men N= 1) (Women N= 1) (Both N= 1), (Multi) vitamin/calcium supplements (Men N= 1) (Women N= 1) (Both N=2), Saturated fat (Both N=1), Processed meat (Both N=1), Milk (Both N=1)
Physical activity**		Physical activity (Men N= 2) (Women N= 1) (Both N= 2)
Hormone replacement therapy***		Hormone replacement therapy (N=3)
Other	(Self-reported) emphysema/ COPD (N=4), Prior diagnosis of pneumonia (N=1), (Occupational) exposure to asbestos (N=2), Environmental tobacco smoke (N=1), Dust exposure (N=1), Prior respiratory disease (N=1), Asthma (N=1)	Previous colorectal cancer screening/colonoscopy (Men N= 1) (Women N= 1) (Both N=2), Serum cholesterol (Men N= 1), IBD (Men N= 2) (Women N= 2) (Both N=1), Previous polyps (Men N= 3) (Women N= 3) (Both N=1)

Arced variables were selected, due to presence in prediction models of multiple cancer types, or due to presence in multiple prediction models of a specific cancer type, and based on availability in the derivation cohort (UCC-SMART cohort) and easy clinical availability.
C-reactive Protein concentration (mg/L) was additionally selected based on previous research showing the relation between CRP and incident (lung) cancer in patients with cardiovascular disease.[15,16]

Prostate	Breast	Gastric
Ethnicity (N=1)	Ethnicity (N=3)	
Deprivation score (N=1)	Education (N=1), Deprivation score (N=1)	Deprivation score (Men N=1) (Women N=1), poor perceived health status (Both N=1), low perceived financial status (Both N=1)
Family history of (prostate) cancer (N=2)	Family history of breast cancer (N=16), Family history of any cancer (N=1)	Family history of gastric cancer (Both N=2)
Meat consumption (N=1)		Consumption of highly salted food (N=1), Drinking tap water (Both N=1)
Physical acitivity (N=1)	Physical activity (N=2)	
	Hormone replacement therapy (N=7)	
Manic depression/ schizophrenia (N=1), hK2 and PSA (N=1)	Age at first live birth (N=16), Age at menopause (N=7), Age at menarche (N=15), birth control pill (N=5), Benign breast disease (BBD)/ atypical hyperplasia (N=8), Manic depression or schizophrenia (N=1), Breast density (N=5), Parity (N=4), Birth index (N=2), Breast biopsy number (N=12), Condom use (N=1), Reproductive age period (N=1), Surgical menopause (N=4)	Barrett's oesophagus (Men N=1) (Women N=1), Peptic ulcer disease (Men N=1) (Women N=1) (Both N=1), Exposed to jobs considered to cause stomach cancer (Both N=1), Eating irregularly and rapidly (Both N=1), H. bacter and pylori antibody and pepsinogen status (N=1)

* Not available in derivation cohort

** Deemed not easily clinically available

*** Considered unfit as predictor, due to heterogeneity in hormone replacement therapies and low proportion of women in cardiovascular disease population.

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Appendix 4. Predictor definitions of UCC-SMART and CANTOS study populations

	UCC-SMART[1]	**CANTOS** [2]
Age	Years, reported by physician/ patient.	Years, reported by physician/ patient.
Sex	Male/female, reported by physician/patient.	Male/female, reported by physician/patient.
Smoking status	Never, former, current. Reported by patient.	Never, former, current. Reported by patient.
History of diabetes mellitus	Either referral diagnosis, self-reported, or a known history of diabetes mellitus at the time of enrolment or a fasting blood glucose ≥7 mmol/L.	Known history of diabetes mellitus ant the time of enrollment.
Weight	Kg, measured at study visit by a study nurse on a standard scale.	Kg, measured at study visit by a study nurse.
Height	Cm, measured at study visit by a study nurse.	Cm, measured at study visit by a study nurse.
Antiplatelet medication use	Yes/no, reported by patient and checked by study nurse with list of medication from pharmacy.	Yes/no, reported by patient and checked by study nurse with list of medication from pharmacy.
C-reactive protein	Mg/L, measured by immunonephelometry (Nephelometer Analyzer BN II, Dade-Behring). From 2013 in heparin plasma on an AU5811 routine chemistry analyzer (Beckman Coulter, Brea, California).	Mg/L, measured by immunoturbidimetry (Roche)
Alcohol use	0, >0-10 per week, >10 per week. Reported by patient.	<1 per week, 1-2 per day, >2 per day. Reported by patient.

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Appendix 5. Incidence rates of cancer and competing events for UCC-SMART and CANTOS study populations

	UCC-SMART		**CANTOS**	
Total study population	N = 7,280		N = 9,322	
Median follow-up time	8.1 years (IQR 4.5-12.1)		3.8 years (IQR 3.2-4.5)	
Cancer type	**Number**	**Incidence rate per 100 PY (95%CI)**	**Number**	**Incidence rate per 100 PY (95%CI)**
Lung cancer	258	0.42 (0.37-0.48)	123 58*	0.35 (0.29-0.42) 0.50 (0.38-0.64*
Colorectal cancer	180	0.29 (0.25-0.34)	72 20*	0.21 (0.16-0.26) 0.17 (0.11-0.27)*
Total cancer	1143	1.97 (1.85-2.08)	509 181*	1.48 (1.35-1.61) 1.57 (1.35-1.82)*
Death				
Total number of deaths	1734	2.81 (2.68-2.94)	988	2.81 (2.64-2.99)
Vascular death	867	1.40 (1.31-1.50)	605	1.72 (1.59-1.87)
Non-cancer death	1108	1.91 (1.80-2.02)	760	2.21 (2.05-2.37)
Non-colorectal cancer death	1649	2.69 (2.57-2.83)	961	2.75 (2.57-2.92)
Non-lung cancer death	1536	2.50 (2.38-2.63)	900	2.57 (2.41-2.74)
Cancer death	451	0.73 (0.66-0.80)	187	0.53 (0.46-0.61)

PY = person years, CI = confidence interval

* Number and incidence rate in patients randomized to placebo in CANTOS trial.

Appendix 6. Interactions with age and transformation of continuous predictors in total, colorectal, and lung cancer models

	Total cancer	**Colorectal cancer**	**Lung cancer**
Event of interest			
Interactions with age	Smoking, CRP	Smoking	CRP, height
Log transformations	-	-	CRP
Quadratic term	CRP	-	-
Competing risk			
Interactions with age	Smoking	CRP, smoking, diabetes	CRP, diabetes, smoking
Log transformations	CRP	CRP	CRP
Quadratic term	Weight	Weight	Weight

Appendix 7. Age-specific baseline survival for colorectal, lung, and total cancer models and corresponding competing events

Age	Total cancer	Non-cancer death	Colorectal cancer	Non-colorectal cancer death	Lung cancer	Non-lung cancer death
45	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
46	1.000000	0.010909	1.000000	0.661636	1.000000	0.482539
47	0.997892	0.052304	1.000000	0.758748	1.000000	0.620883
48	0.997061	0.370462	1.000000	0.904212	1.000000	0.844101
49	0.998296	0.223464	1.000000	0.857343	1.000000	0.771617
50	0.998671	0.321635	1.000000	0.831196	0.999808	0.734537
51	0.998565	0.066126	0.998930	0.666761	0.999697	0.556163
52	0.998545	0.103195	1.000000	0.673453	0.999879	0.606676
53	0.997809	0.291191	1.000000	0.774060	0.999805	0.742313
54	0.996838	0.354311	0.998779	0.854106	0.999917	0.816470
55	0.996616	0.080358	0.999456	0.650916	0.999857	0.505876
56	0.997228	0.068010	0.996599	0.617418	0.999758	0.470679
57	0.996627	0.131837	0.998243	0.670176	0.999678	0.581502
58	0.995940	0.146715	0.998433	0.616549	0.999666	0.590496
59	0.996709	0.095736	0.998176	0.573117	0.999476	0.499736
60	0.997872	0.220404	0.998997	0.648761	0.999718	0.585399
61	0.996867	0.205495	0.999069	0.555074	0.999736	0.541788
62	0.996742	0.216696	0.999131	0.586818	0.999900	0.519124
63	0.995943	0.157539	0.998931	0.543106	0.999537	0.496529
64	0.996522	0.156689	0.998711	0.466949	0.999677	0.412636
65	0.996691	0.330062	0.998560	0.543395	0.999696	0.535206
66	0.995958	0.212372	0.998391	0.469155	0.999744	0.413736
67	0.996804	0.131099	0.999138	0.428482	0.999738	0.392860
68	0.996817	0.130555	0.997440	0.376002	0.999778	0.387324
69	0.996121	0.095067	0.998756	0.322198	0.999770	0.276167
70	0.996754	0.182875	0.998977	0.382799	0.999730	0.382724
71	0.996442	0.080557	0.998378	0.268446	0.999761	0.257421
72	0.996512	0.188507	0.998816	0.328136	0.999714	0.329868
73	0.997035	0.088450	0.998826	0.253319	0.999849	0.233621
74	0.996679	0.144884	0.997420	0.264050	0.999802	0.287865
75	0.996777	0.140345	0.998408	0.244778	0.999895	0.281182
76	0.996504	0.127645	0.997813	0.176633	0.999900	0.191156
77	0.997465	0.083942	0.997973	0.135055	0.999811	0.152363

Appendix 7. Continued

Age	Total cancer	Non-cancer death	Colorectal cancer	Non-colorectal cancer death	Lung cancer	Non-lung cancer death
78	0.997110	0.132351	0.997876	0.151134	0.999795	0.200271
79	0.997612	0.075976	0.998424	0.102477	0.999836	0.117197
80	0.997660	0.068319	0.999251	0.059011	0.999901	0.079315
81	0.997412	0.079869	0.997713	0.068455	0.999890	0.087441
82	0.998449	0.043326	0.998962	0.044134	0.999975	0.048844
83	0.998973	0.032979	0.999633	0.031685	0.999943	0.046864
84	0.998897	0.029180	0.999093	0.019001	0.999964	0.024884
85	0.998123	0.015178	1.000000	0.003899	0.999880	0.012949
86	0.998436	0.028230	0.998703	0.006675	0.999903	0.009146
87	0.999413	0.015374	1.000000	0.002519	0.999932	0.006293
88	0.999589	0.033008	1.000000	0.019628	1.000000	0.036531
89	0.999485	0.051207	1.000000	0.021783	0.999902	0.046287

Appendix 8. Subdistribution hazard ratios and shrinkage factors for total cancer, colorectal cancer, and lung cancer model

A. Total cancer

Total cancer	Hazard ratio	Lower limit 95% CI	Upper limit 95% CI	**Competing risk (non-cancer death)**	Hazard ratio	Lower limit 95% CI	Upper limit 95% CI
Male sex	0.9685	0.8056	1.1644	Male sex	1.6001	1.3235	1.9345
Former smoker	1.0559	0.8874	1.2565	Former smoker	0.8929	0.7372	1.0816
Current smoker	1.1908	0.9918	1.4297	Current smoker	1.2009	0.9952	1.4490
Weight	0.9955	0.9902	1.0009	Weight	0.8975	0.8658	0.9304
CRP	0.9827	0.8287	1.1654	Weight^2	1.0006	1.0004	1.0008
CRP^2	1.0018	0.9966	1.0071	log(CRP)	1.5613	1.4596	1.6700
Former smoker*age¥	1.0502	1.0348	1.0658	Former smoker*age¥	1.0852	1.0713	1.0993
Current smoker*age¥	1.0602	1.0428	1.0778	Current smoker*age¥	1.0952	1.0807	1.1099
Height	1.0126	1.0027	1.0226	Height	0.9923	0.9824	1.0023
Diabetes mellitus	0.8855	0.7579	1.0346	Diabetes mellitus	1.6865	1.4705	1.9342
Alcohol >0-10 per week	0.9308	0.7876	1.1000	Alcohol >0-10 per week	0.8138	0.6946	0.9535
Alcohol >10 per week	1.0425	0.8681	1.2519	Alcohol >10 per week	0.8210	0.6875	0.9805
Antiplatelet use	0.9202	0.8058	1.0508	Antiplatelet use	0.7384	0.6500	0.8387
CRP*age	1.0007	0.9980	1.0034				
(CRP^2)*age	1.0000	0.9999	1.0000				
Shrinkage factor 0.91				Shrinkage factor 0.98			

Appendix 8. Subdistribution hazard ratios and shrinkage factors for total cancer, colorectal cancer, and lung cancer model

B. Colorectal cancer

Colorectal cancer	Hazard ratio	Lower limit 95% CI	Upper limit 95% CI	**Competing risk (non colorectal cancer death)**	Hazard ratio	Lower limit 95% CI	Upper limit 95% CI
Male sex	0.7216	0.4583	1.1363	Male sex	1.6496	1.4050	1.9369
Former smoker	0.9180	0.6046	1.3940	Former smoker	1.3961	1.1784	1.6540
Current smoker	0.8836	0.5617	1.3899	Current smoker	2.0992	1.7792	2.4767
Weight	1.0069	0.9938	1.0202	Weight	0.8732	0.8467	0.9005
CRP	0.9587	0.9264	0.9922	Weight^2	1.0008	1.0006	1.0009
Former smoker*age¥	1.0529	1.0205	1.0864	log(CRP)	1.1747	0.8730	1.5805
Current smoker*age¥	1.0385	1.0007	1.0776	Diabetes mellitus	3.9731	1.6066	9.8253
Height	1.0018	0.9771	1.0271	Height	1.0059	0.9975	1.0144
Diabetes mellitus	1.0109	0.6955	1.4691	Diabetes*age	0.9853	0.9719	0.9989
Alcohol >0-10 per week	1.0796	0.7034	1.6569	Alcohol >0-10 per week	0.8821	0.7710	1.0092
Alcohol >10 per week	1.2117	0.7543	1.9464	Alcohol >10 per week	0.9556	0.8247	1.1073
Antiplatelet use	1.0753	0.7579	1.5256	Antiplatelet use	0.7671	0.6908	0.8518
				Former smoker*age¥	1.0060	0.9938	1.0184
				Current smoker*age¥	1.0133	1.0006	1.0261
				log(CRP)*age	1.0029	0.9996	1.0063
Shrinkage factor 0.71				Shrinkage factor 0.98			

C. Lung cancer

Lung cancer	Hazard ratio	Lower limit 95% CI	Upper limit 95% CI	**Competing risk (non lung cancer death)**	Hazard ratio	Lower limit 95% CI	Upper limit 95% CI
Male sex	1.1138	0.7527	1.6482	Male sex	1.5790	1.3402	1.8604
Former smoker	3.3872	1.7610	6.5152	Former smoker	1.0978	0.9293	1.2970
Current smoker	7.2624	3.7836	13.9399	Current smoker	1.3545	1.1499	1.5955
Weight	0.9833	0.9722	0.9946	Weight	0.8951	0.8674	0.9238
Log(CRP)	0.7000	0.3157	1.5520	Weight^2	1.0006	1.0004	1.0008
Log(CRP)*age	1.0080	0.9986	1.0176	Log(CRP)	0.9456	0.6956	1.2856
Height*age	1.0003	1.0002	1.0005	Diabetes mellitus	4.4843	1.7888	11.2412
Height	0.9954	0.9734	1.0180	Height	1.0004	0.9918	1.0091
Diabetes mellitus	0.6860	0.4758	0.9891	Log(CRP)*age	1.0046	1.0012	1.0081
Alcohol >0-10 per week	1.1822	0.8015	1.7436	Diabetes*age	0.9838	0.9703	0.9975
Alcohol >10 per week	1.3343	0.8853	2.0110	Alcohol >0-10 per week	0.8491	0.7404	0.9739
Antiplatelet use	0.7787	0.5992	1.0119	Alcohol >10 per week	0.8876	0.7629	1.0327
				Antiplatelet use	0.8332	0.7464	0.9300
				Former smoker*age¥	1.0262	1.0138	1.0388
				Current smoker*age¥	1.0355	1.0224	1.0487
Shrinkage factor 0.93				Shrinkage factor 0.97			

¥ For the interaction between smoking and age, age was centered around the mean.

Appendix 9. Cancer model formulas for total cancer, colorectal cancer, and lung cancer

A. Total cancer model

Total cancer model

1-year survival = (age-specific 1-yr baseline survival[¥])^exp(A) [†]

A = -0.0290 (if male) - 2.6865 (if former smoker) [§] - 3.1078 (if current smoker) [§] + 0.0443*age (if former smoker) + 0.0529*age (if current smoker) - 0.0041*weight (kg) - 0.0158*CRP (mg/L) + 0.0017*CRP^2 (mg/L) + 0.00006*age*CRP (mg/L) + 0.0000*age*(CRP^2) - 0.1101 (if history of diabetes mellitus) + 0.0113*height (cm) - 0.0650 (if alcohol use >0-10 per week) [§] + 0.0377 (if alcohol use >10 per week) [§] - 0.0754 (if antiplatelet medication use) + 0.0392 (if randomized to Canakinumab 50mg) - 0.1863 (if randomized to Canakinumab 150mg) - 0.1393 (if randomized to Canakinumab 300mg)

Mortality not due to cancer - model

1-year survival = (age-specific 1-yr baseline survival[¥])^exp(B) [†]

B = 0.4624 (if male) - 5.073 (if former smoker) [§] - 5.3398 (if current smoker) [§] + 0.0804*age (if former smoker) + 0.0895*age (if current smoker) - 0.1064*weight (kg) + 0.0006*weight^2 (kg) + 0.4383*CRP (mg/L) + 0.5142 (if history of diabetes mellitus) - 0.0076*height (cm) - 0.2027 (if alcohol use >0-10 per week) [§] - 0.1940 (if alcohol use >10 per week) [§] - 0.2984 (if antiplatelet medication use) - 0.0726 (if randomized to Canakinumab 50mg) - 0.0619 (if randomized to Canakinumab 150mg) + 0.0296 (if randomized to Canakinumab 300mg)

[†] *Estimates after adjusting for shrinkage factor are shown*

[¥] *Age-specific baseline survivals are shown in Online table S7.*

[§] *The coefficients for alcohol use and smoking status should not be added up.*

B. Colorectal cancer model

Colorectal cancer model

1-year survival = (age-specific 1-yr baseline survival[¥])^exp(A) [†]

A = -0.2304 (if male) - 2.3084 (if former smoker) [§] - 1.7321 (if current smoker) [§] + 0.0364*age (if former smoker) + 0.0267*age (if current smoker) + 0.0048*weight (kg) - 0.0298*CRP (mg/L) + 0.0076 (if history of diabetes mellitus) + 0.0013*height (cm) + 0.0541 (if alcohol use >0-10 per week) [§] + 0.1356 (if alcohol use >10 per week) [§] + 0.0513 (if antiplatelet medication use) + 0.0488 (if randomized to Canakinumab 50mg) + 0.039221 (if randomized to Canakinumab 150mg) + 0.2852 (if randomized to Canakinumab 300mg)

Mortality not due to colorectal cancer - model

1-year survival = (age-specific 1-yr baseline survival[¥])^exp(B) [†]

B = 0.4894 (if male) - 0.0361 (if former smoker) [§] - 0.0692 (if current smoker) [§] + 0.0059*age (if former smoker) + 0.0129*age (if current smoker) - 0.1326*weight (kg) + 0.0007*weight^2 (kg) + 0.1574*log(CRP) (mg/L) + 0.0029*age*log(CRP) + 1.3489 (if history of diabetes mellitus) - 0.0145*age (if history of diabetes mellitus) + 0.0057*height (cm) - 0.1227 (if alcohol use >0-10 per week) [§] - 0.0444 (if alcohol use >10 per week) [§] - 0.2593 (if antiplatelet medication use) - 0.0726 (if randomized to Canakinumab 50mg) - 0.0305 (if randomized to Canakinumab 150mg) + 0.0488 (if randomized to Canakinumab 300mg)

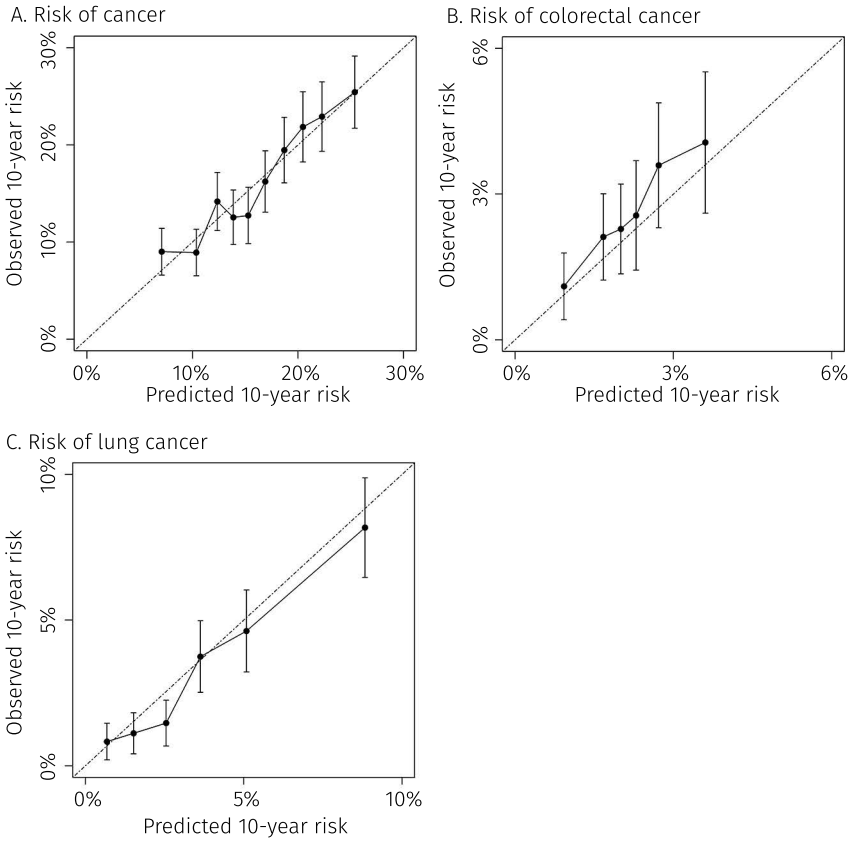
[†] *Estimates after adjusting for shrinkage factor are shown*

[¥] *Age-specific baseline survivals are shown in Online table S7.*

[§] *The coefficients for alcohol use and smoking status should not be added up.*

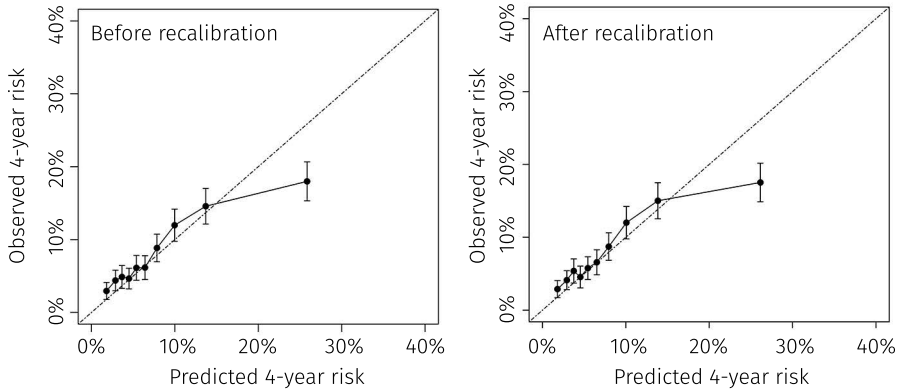
Appendix 9. Continued

C. Lung cancer model
Lung cancer model
1-year survival = (age-specific 1-yr baseline survival¥)^exp(A)†
A = 0.1007 (if male) + 1.1395 (if former smoker)§ + 1.8519 (if current smoker)§ - 0.0157*weight (kg) + 0.0075*log(CRP) (mg/L) + 0.0003*age*log(CRP) (mg/L) - 0.3520 (if history of diabetes mellitus) - 0.0043*height (cm) + 0.0003*age*height (cm) + 0.1563 (if alcohol use >0-10 per week)§ + 0.2694 (if alcohol use >10 per week)§ - 0.2336 (if antiplatelet medication use) - 0.2614 (if randomized to Canakinumab 50mg) - 0.4780 (if randomized to Canakinumab 150mg) – 1.1394 (if randomized to Canakinumab 300mg)
Mortality not due to lung cancer - model
1-year survival = (age-specific 1-yr baseline survival¥)^exp(B)†
B = 0.4436 (if male) - 1.4602 (if former smoker)§ - 1.7933 (if current smoker)§ + 0.0251*age (if former smoker) + 0.0338*age (if current smoker) - 0.1076*weight (kg) + 0.0006*weight^2 (kg) - 0.0543*log(CRP) (mg/L) + 0.0046*age*log(CRP) (mg/L) + 1.4572 (if history of diabetes mellitus) - 0.0159*age (if history of diabetes mellitus) + 0.0004*height (cm) - 0.1588 (if alcohol use >0-10 per week)§ - 0.1158 (if alcohol use >10 per week)§ - 0.1773 (if antiplatelet medication use) - 0.0513 (if randomized to Canakinumab 50mg) - 0.0726 (if randomized to Canakinumab 150mg) + 0.0101 (if randomized to Canakinumab 300mg)
† *Estimates after adjusting for shrinkage factor are shown*
¥*Age-specific baseline survivals are shown in Online table S7.*
§ *The coefficients for alcohol use and smoking status should not be added up.*

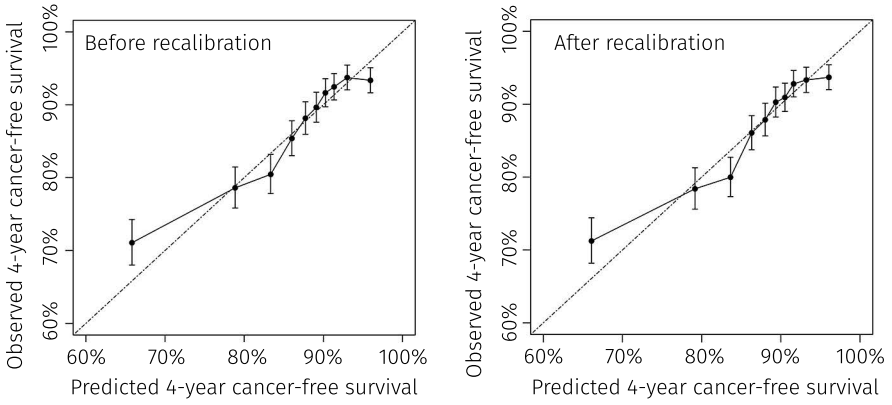


Appendix 10. Internal calibration plots of predicted and observed risk of total, colorectal, and lung cancer in the UCC-SMART data

A. Risk of death not due to cancer



B. Cancer-free survival (combined model)

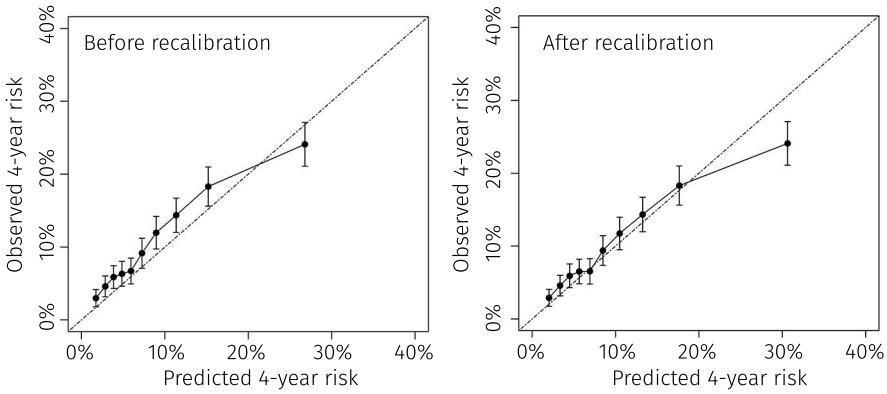


Appendix 11. External calibration plots of non-cancer mortality and cancer-free survival before and after recalibration in the CANTOS data

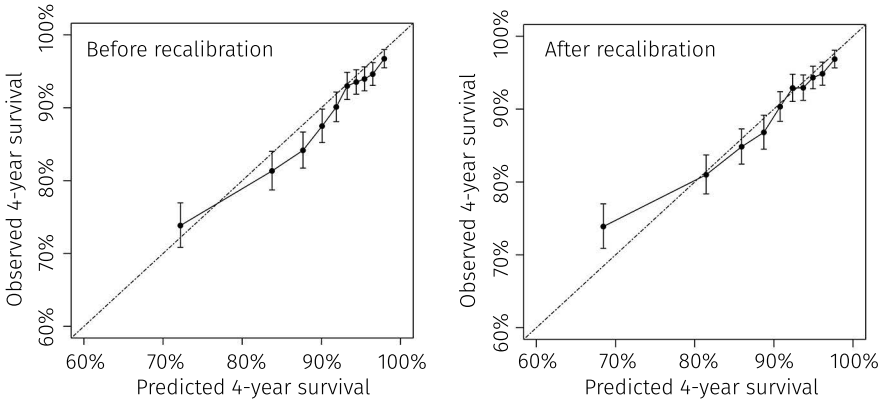
A. Calibration plots are shown of the predicted versus observed 4-year risk of non-cancer mortality (competing risk of total cancer) in the CANTOS study population, before and after recalibration. C-statistic is 0.67 (95%CI 0.65-0.69)

B. Calibration plots are shown of the predicted versus observed 4-year cancer-free survival, which is the probability of being healthy (no cancer) and alive (no competing event). C-statistic is 0.66 (95%CI 0.64-0.67)

A. Risk of death not due to colorectal cancer



B. Colorectal cancer-free survival (combined model)

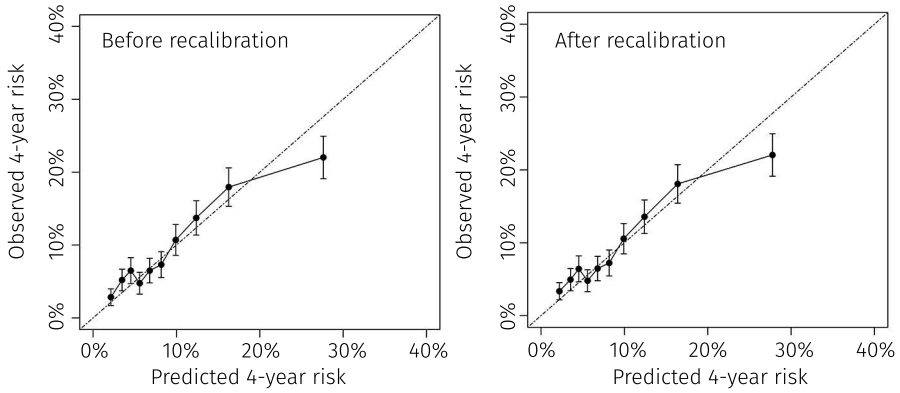


Appendix 12. External calibration plots of non-colorectal cancer death and colorectal cancer-free survival before and after recalibration in the CANTOS data

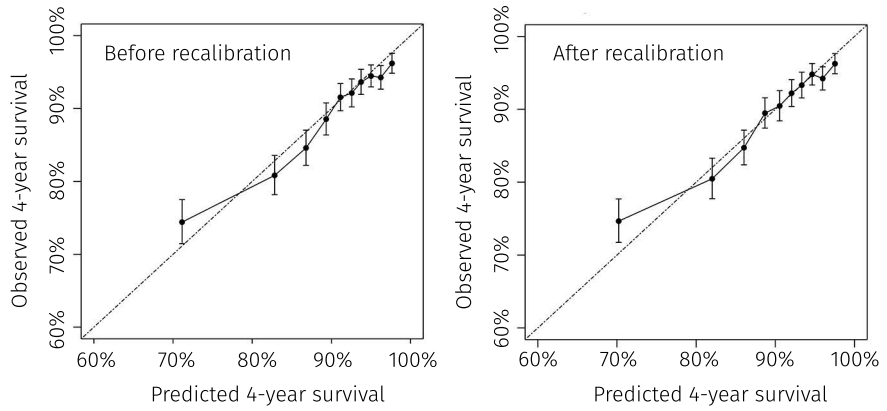
A. Calibration plots are shown of the predicted versus observed 4-year risk of non-colorectal cancer mortality (competing risk of colorectal cancer) in the CANTOS study population, before and after recalibration. C-statistic is 0.68 (95%CI 0.67-0.70)

B. Calibration plots are shown of the predicted versus observed 4-year colorectal cancer-free survival, which is the probability of being healthy (no colorectal cancer) and alive (no competing event). C-statistic is 0.68 (95%CI 0.66-0.70)

A. Risk of death not due to lung cancer



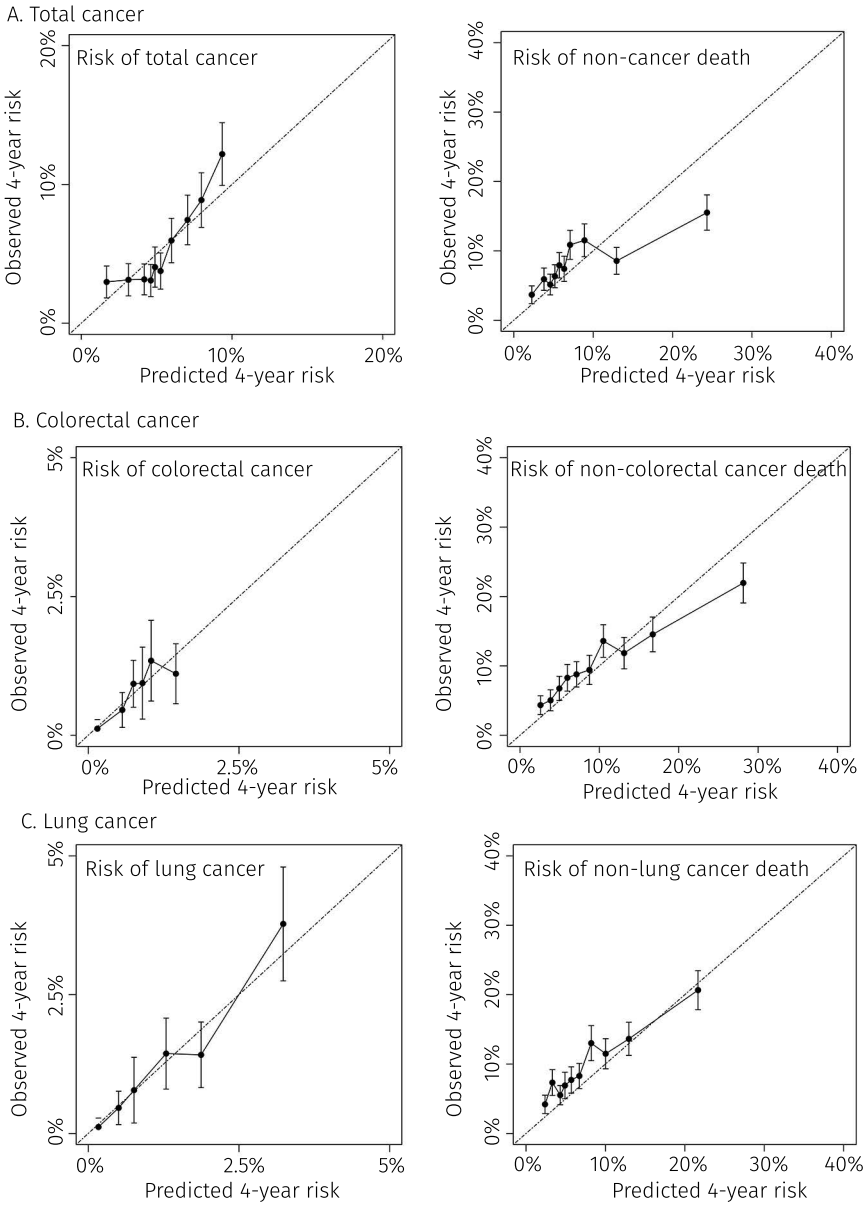
B. Lung cancer-free survival (combined model)



Appendix 13. External calibration plots of non-lung cancer death and lung cancer-free survival before and after recalibration in the CANTOS data

A. Calibration plots are shown of the predicted versus observed 4-year risk of non-lung cancer mortality (competing risk of lung cancer) in the CANTOS study population, before and after recalibration. C-statistic is 0.68 (95%CI 0.66-0.70)

B. Calibration plots are shown of the predicted versus observed 4-year lung cancer-free survival, which is the probability of being healthy (no lung cancer) and alive (no competing event). C-statistic is 0.68 (95% CI 0.66-0.69)



Appendix 14. External calibration plots for total, colorectal, and lung cancer risk and competing risks of the simple model after recalibration in the CANTOS data

Calibration plots are shown of the predicted versus observed 4-year risk of total cancer, colorectal cancer, and lung cancer in the CANTOS study population after recalibration. In this simple model, only sex and smoking status were included as predictors (age is the underlying time scale). The study population is divided into quantiles based on the predicted risk, and ordered according to increasing predicted risk. The diagonal dotted line represents perfect calibration.

Appendix 15. Median predicted 10-year and lifetime risks in 5-year age groups for UCC-SMART and CANTOS study populations

A. UCC-SMART study population

	Total cancer		**Colorectal cancer**		**Lung cancer**	
Age group (5-year interval)	Median (range) predicted 10-year risk (%)	Median (range) predicted lifetime risk (%)	Median (range) predicted 10-year risk (%)	Median (range) predicted lifetime risk (%)	Median (range) predicted 10-year risk (%)	Median (range) predicted lifetime risk (%)
45-49	8 (4-14)	45 (21-59)	1 (0-2)	6 (1-11)	1 (0-7)	10 (1-31)
50-54	12 (7-19)	44 (12-58)	2 (0-3)	6 (1-11)	3 (0-12)	10 (1-32)
55-59	16 (7-23)	41 (10-57)	2 (0-3)	6 (0-11)	4 (0-17)	9 (1-28)
60-64	19 (5-30)	37 (6-53)	2 (0-3)	5 (1-10)	4 (0-20)	7 (1-28)
65-69	21 (4-32)	32 (5-47)	3 (0-5)	5 (0-8)	4 (0-19)	6 (1-24)
70-74	21 (3-31)	26 (4-39)	3 (0-5)	4 (0-7)	3 (0-18)	4 (0-18)
75-80	16 (2-28)	17 (2-30)	2 (0-5)	2 (0-5)	3 (0-13)	3 (0-14)

B. CANTOS study population

	Total cancer		**Colorectal cancer**		**Lung cancer**	
Age group (5-year interval)	Median (range) predicted 10-year risk (%)	Median (range) predicted lifetime risk (%)	Median (range) predicted 10-year risk (%)	Median (range) predicted lifetime risk (%)	Median (range) predicted 10-year risk (%)	Median (range) predicted lifetime risk (%)
45-49	6 (3-11)	33 (7-52)	1 (0-2)	5 (1-13)	1 (0-7)	7 (0-37)
50-54	10 (5-17)	32 (8-51)	2 (0-3)	5 (1-13)	2 (0-12)	7 (0-35)
55-59	12 (5-20)	30 (7-50)	2 (0-4)	5 (0-13)	3 (0-18)	6 (0-36)
60-64	15 (4-25)	27 (6-48)	2 (0-4)	4 (0-12)	3 (0-24)	5 (0-34)
65-69	16 (3-29)	22 (4-40)	2 (0-6)	4 (0-10)	3 (0-21)	4 (0-27)
70-74	15 (2-31)	18 (2-35)	3 (0-6)	3 (0-8)	2 (0-17)	3 (0-19)
75-80	10 (1-23)	11 (1-24)	2 (0-5)	2 (0-5)	2 (0-13)	2 (0-14)

Chapter 8

General discussion

General discussion

In this thesis, risk factors and risk prediction beyond the scope of current usual care are investigated in patients with stable cardiovascular disease (CVD), by means of three main topics: Systemic low-grade inflammation, cardiovascular calcification, and individualized risk prediction.

Key findings

In this thesis it was shown that,

1. Systemic low-grade inflammation, measured by CRP, is a risk factor for recurrent cardiovascular events as well as incident cancer, markedly lung cancer, in patients with stable CVD.
2. Healthy lifestyle changes; smoking cessation, weight loss, and increase in physical activity reduce systemic low-grade inflammation, measured by CRP, in patients with stable CVD.
3. Differences in associations between risk factors and calcification at various anatomical locations stress the divergence in pathophysiological pathways. Coronary artery calcium (CAC) scores are most strongly related to a combined endpoint of recurrent cardiovascular events and cardiovascular interventions, independent of traditional risk factors, and independent of heart valve and thoracic aorta calcification scores in patients with stable CVD.
4. Addition of CAC scores improved model performance of a risk score based on traditional risk factors, for the prediction of a combined endpoint of recurrent major cardiovascular events and cardiovascular interventions (MACE+) in patients with stable CVD. Addition of TAC or heart valve calcium scores did not improve risk predictions.
5. The 10-year combined risk of recurrent major cardiovascular events and cardiovascular interventions (MACE+) can be estimated in patients with established coronary heart disease or cerebrovascular disease. However, cardiovascular interventions in patients with peripheral artery disease can not be predicted reliably with the developed risk model (extended SMART risk score).
6. Lifetime and 10-year risk of total, colorectal, and lung cancer can be estimated with easily clinically available predictors in patients with established CVD, showing a wide distribution of predicted lifetime risks for total cancer and lung cancer.

Part I Systemic low-grade inflammation

Inflammation as a treatment target in patients with stable CVD

The etiologic relation between low-grade systemic inflammation and atherosclerotic disease,[1] as was also observed in **chapter 2**, has led to the hypothesis that inflammation may be an effective treatment target in patients with established CVD to prevent second events.[1] In fact, the CANTOS trial has shown that by lowering inflammation with canakinumab, the risk of recurrent cardiovascular events was lowered with a hazard ratio (HR) of 0.85 (95% confidence interval (CI) 0.74-0.98) for the 150 mg canakinumab dosage group.[2] The results of this landmark trial proved that inflammation is indeed involved in the pathogenesis of atherosclerotic disease, and showed that lowering inflammation pharmaceutically reduced recurrent cardiovascular event rates in patients with stable CVD and with elevated inflammatory markers (C-reactive protein (CRP) ≥ 2 mg/L). Canakinumab, however, was deemed not the right pharmaceutical option for this indication. The incremental cost-effectiveness ratio of canakinumab (at the 2019 market price of $73,000 per year) was estimated at $6.4 million per quality-adjusted life-year (QALY), or $3.5 million per QALY when taking the beneficial effect of canakinumab on lung cancer incidence into account, and exceeds the $100,000 per QALY willingness-to-pay threshold.[3] Furthermore, the positive effect was considered modest, and it was debated whether canakinumab would be effective in all patients with coronary heart disease. The registration of canakinumab for this indication was halted by Novartis,[4] and thus canakinumab will not become available as part of secondary prevention treatment strategies.

Low-dose methotrexate was considered an alternative, inexpensive approach to inhibition of inflammation in patients with stable CVD. However, the Cardiovascular Inflammation Reduction Trial (CIRT) showed that methotrexate did not reduce inflammatory markers, nor result in fewer recurrent cardiovascular events compared to placebo.[5] Colchicine, an inexpensive, widely used, potent anti-inflammatory drug, lowered the incidence of a combined endpoint of vascular death, resuscitated cardiac arrest, myocardial infarction, stroke, and urgent hospitalization for angina leading to coronary revascularization with a HR of 0.77 (95%CI 0.61-0.96) compared to placebo in the Colchicine Cardiovascular Outcomes Trial (COLCOT).[6] The Low-Dose Colchicine (LoDoCo) trial had previously shown a positive effect of colchicine in a prospective, randomized, observer-blinded endpoint (PROBE) trial setting, with a HR of 0.33 (95%CI 0.18-0.59) for the composite primary outcome of acute coronary syndrome, out-of-hospital cardiac arrest, or noncardioembolic ischemic stroke, but the trial was not placebo controlled.[7] The LoDoCo2 trial, a randomized placebo-controlled trial investigating colchicine in patients with stable coronary artery disease, is currently ongoing.[8]

Systemic low-grade inflammation is a promising treatment target in patients with established CVD to prevent second events, and currently colchicine might be the most likely contender for this indication, in anticipation of LoDoCo2 trial results. Treatment of systemic inflammation

could become 'the fourth pillar' of medical management in patients with stable CVD, in addition to lipid lowering treatment, blood pressure control, and anti-thrombotic therapy. However, questions remain concerning side-effects, costs, and long term effects of colchicine, as well as the selection of patients with established CVD who will qualify for pharmaceutical lowering of inflammation.

Who to treat with anti-inflammatory therapy?

CANTOS, COLCOT, and LoDoCo(2) included patients with coronary heart disease (CHD), and it is not yet certain whether results are generalizable to all patients with CVD. The relation between CRP and cardiovascular events was previously observed in patients with peripheral artery disease (PAD),[9] in patients with cerebrovascular disease (CeVD),[10] and in patients with CHD, PAD, and CeVD combined (**chapter 2**). The pathophysiological mechanism of inflammation in atherosclerotic disease is systemic, and not limited to coronary arteries.[1,11] It is therefore likely that patients with all types of established CVD could benefit from anti-inflammatory treatment, but this should be confirmed in future studies. The Colchicine for Prevention of Vascular Inflammation in Non-cardio Embolic Stroke (CONVINCE) trail, investigating the effect of colchicine in patients with established CeVD for the prevention of major cardiovascular events is currently ongoing (clinical trial number NCT02898610).

Similar to blood pressure lowering and lipid lowering therapy, selecting patients for anti-inflammatory treatment on the basis of inflammatory markers, such as CRP, seems rational. For cardiovascular risk assessment, CRP thresholds of <1 mg/L, 1-3 mg/L, and >3 mg/L,[12] or <2 mg/L and >2 mg/L[13] are commonly used. The CANTOS trial only included patients with CRP plasma levels ≥ 2 mg/L.[14] In **chapter 2**, the median CRP level was 1.8 mg/L (IQR 0.9-3.54) in patients with stable CVD. Furthermore, a relation between CRP quintiles and recurrent cardiovascular events was observed from the fourth CRP quintile (HR 1.34 (95%CI 1.11-1.62)) with a median CRP level in that quintile of 3.1 mg/L (range 2.3-4.1), whereas the median CRP level in the third quintile was 1.8 mg/L (range 1.4-2.3) with a HR of 1.11 (95%CI 0.92-1.35). These results suggest that a CRP level of 2.0 mg/L could be a sensible treatment threshold. The COLCOT and the LoDoCo(2) trial, however, did not select patients on the basis of plasma levels of inflammatory biomarkers. A prespecified subgroup analysis embedded in a trial, studying the beneficial effects of anti-inflammatory medication in patients with CRP levels < 2 mg/L and ≥ 2 mg/L at the start of treatment could provide further insight.

An additional strategy for the selection of patients with stable CVD that will benefit from anti-inflammatory treatment, could be based on risk and benefit predictions for an individual patient. Decision support tools estimating the risk of recurrent cardiovascular events and benefit from therapy expressed in healthy life-years gained – as are currently available for lipid lowering, blood pressure lowering, antiplatelet therapy, and additional anti-thrombotic treatment

schemes[15-18] (www.U-Prevent.com) – are not available for anti-inflammatory therapy. In future, such models could be developed, for example for colchicine with treatment effect estimates from COLCOT or LoDoCo2, to estimate treatment benefit from anti-inflammatory therapy in addition to traditional preventive therapies.

Effects of anti-inflammatory therapy on lung cancer incidence

The CANTOS trial[19] and **chapter 2** showed that inflammation is also a risk factor for cancer, markedly lung cancer. The relative effect of canakinumab on the incidence of lung cancer was quite substantial in the CANTOS trial, with a HR of 0.55 (0.39–0.78) comparing all dosage groups to placebo,[19] and in **chapter 2** is was shown that per 1 mg/L higher CRP, the HR was 1.16 (95% CI 1.10-1.22) for incident lung cancer. Even though the relative effect of Canakinumab might be substantial, the absolute number of lung cancers that are prevented by anti-inflammatory therapy will be limited, and thus the number needed to treat high (estimated 124). Anti-inflammatory therapy will therefore aim to reduce the risk of recurrent cardiovascular events, and will not be given as preventive lung cancer treatment in all patients with stable CVD. However, it might have a positive 'side-effect' by reducing the incidence of lung cancer (mortality). The population of patients with established CVD is growing, with an estimated number of 1.55 million in the Netherlands in 2018[20], and these patients are at a higher risk of lung cancer (SIR 1.56 (95%CI 1.31-1.83) compared to the general population)[21]. Imagine half of the patients with stable CVD, about 775,000 patients, were treated with anti-inflammatory medication. Using the incidence rate (IR) from the CANTOS trial for the treatment groups (IR 0.27 per 100 person years) and from the placebo group (IR 0.49 per 100 person years), it could be estimated that if 775,000 patients are treated for a year, 1706 fewer lung cancers would be diagnosed per treatment year. This is a rough estimation with several assumptions, including the assumption that any anti-inflammatory drug will have the same effect on lung cancer incidence as canakinumab, and without taking potential treatment harms into account, but shows that anti-inflammatory therapy in patients with established CVD could be beneficial through multiple courses of action.

Lifestyle changes affect systemic inflammation in patients with stable CVD

As part of secondary cardiovascular prevention, guidelines include recommendations for a healthy lifestyle, including no tobacco smoke exposure, sufficient physical exercise, and a healthy weight.[22] Selecting patients for anti-inflammatory therapy based on CRP levels ≥ 2 mg/L and/or based on the highest predicted risks and treatment benefits, will also select patients with unhealthy lifestyle habits. **Chapter 3** showed that smoking and a higher weight are associated with elevated CRP concentrations, and that smoking cessation, weight loss, and increase of physical exercise reduce CRP concentrations in patients with stable CVD. In CANTOS 23% to 25% of the enrolled patients were current smokers with a median body mass index (BMI) of 30 kg/m^2 (IQR 27-34) in all separate treatment arms,[2] in COLCOT the percentage

of current smokers was 30% and the median BMI 28 kg/m^2 (standard deviation (SD) ±5) for both the colchicine and placebo group.[6] It could be argued that patients should optimize weight and quit smoking first, before meeting requirements for anti-inflammatory medication. However, **chapter 3** also showed that, although 50% of the smokers did quit smoking during follow-up, the other 50% did not, and only 29% of the patients with stable CVD had achieved a body mass index (BMI) ≤ 25 kg/m^2. Setting lifestyle requirements before receiving pharmaceutical treatment is not feasible and would encounter ethical objections and dilemmas such as cut-off values, exceptional cases, and the comparison to other cardiovascular treatments that are given to patients who have not optimized their lifestyle. However, it could be argued that increasing attention for lifestyle optimization is needed, and that providing earlier and more assistance might help patients to change unhealthy lifestyle habits.

The recent COVID-19 outbreak emphasizes the importance of a healthy lifestyle in patients with stable CVD. Patients with COVID-19 infection and pre-existing CVD have an increased risk of severe disease and death[23-26] (RR 3.30; 95%CI 2.03-5.36 comparing the proportion of patients with established CVD in severely ill patients compared to patients with milder disease).[24] Hypothesized pathological pathways are through risk factors for CVD that also influence immune function, such as age, diabetes, and hyperlipidemia.[23] Furthermore, obesity is considered an important risk factor for severe COVID-19 infection, through several mechanisms, including reduction of protective cardiorespiratory reserve, immune dysregulation, and enhancement of thrombosis.[27] These observations stress the importance of a healthy lifestyle in patients with established CVD, not only to prevent recurrent cardiovascular events and cancer, but also to prevent a fulminant course of COVID-19 infection, and potentially other infectious diseases.

Previous lifestyle intervention trials provided unsatisfactory (long-term) results,[28-31] and other methods to help patients optimize lifestyle behaviors are needed. Individual predictions of risks for cancer and recurrent cardiovascular events by the models of **chapter 6** and **chapter 7** could also be used in clinical practice to emphasize healthy lifestyle changes. Although most patients will be aware that smoking and being obese is unhealthy, and clinicians will educate patients about benefits of a healthy lifestyle, quantifying personalized risks for the individual patient could increase awareness and potentially motivate patients to change lifestyle behaviors. Previous studies found that personalized risk assessment was considered informative and motivating by patients,[32] and that effective risk communication can lead to changes in behavior,[33] although the effects were small and inconsistent,[34] and dependent on representation of risk information.[33] The positive effects of lifestyle on cancer incidence are possibly underemphasized compared to the effects of lifestyle on cardiovascular disease, even though an estimated maximum of 60% of cancer deaths in the United States may be attributable to lifestyle and environmental factors (tobacco smoke, alcohol intake, obesity, physical inactivity, occupations, infectious agents, and exposure to ionizing and solar radiation).[35] Emphasizing lifestyle behaviors by discussing

cardiovascular risk as well as cancer risk could be more effective, as some patients might be more motivated by cancer prevention, and others by cardiovascular prevention.[36] Quantifying personal benefits from healthy lifestyle changes might give even more insight, however, defining effects of lifestyle changes, such as smoking cessation, physical activity increase, weight loss, and diet, will be challenging, especially for physical activity and dietary habits.

Part II Cardiovascular calcification

Cardiovascular calcium scores and cardiovascular risk prediction

Further improvement of existing risk scores is warranted to maximize prediction accuracy, for example by studying prognostic value of additional predictors. Coronary artery calcium has been extensively studied as an additional predictor in primary prevention research. In current European primary prevention guidelines, it is recommended to use available coronary artery calcium (CAC) scores to reclassify patients with calculated SCORE risks around the 5% or 10% treatment thresholds.[22] Limited evidence is available in patients with established CVD. **Chapter 5** indicates that addition of CAC scores improves model performance for the prediction of MACE+, compared to a risk model with traditional atherosclerotic risk factors. This finding leads to the recommendation to implement CAC scores in risk prediction models for patients with established CVD, and to use these models when CAC scores are available for an individual patient. Taking the relatively low available number of events for analysis (N=77) into account in **chapter 5**, this is a preliminary recommendation. As treatment thresholds are not available for patients with stable CVD, risk reclassification by the model with addition of CAC scores, as calculated by the net reclassification index (NRI), is more difficult to interpret and conclusions on the clinical relevance are less straightforward. The maximum change in predicted 4-year risk of MACE+ for patients with a follow-up duration of at least 4 years comparing the traditional model to the model with addition of CAC scores was 10% (10% higher predicted risk by the model with CAC) in patients with an event, and -17% for control patients without an event (17% lower predicted risk by addition of CAC scores to the model). Despite the absence of treatment thresholds, a lower predicted risk of 17% or a higher predicted risk of 10% 4-year risk, could justify treatment intensification or refraining from additional therapy for an individual patient.

There is insufficient evidence for the recommendation to actively acquire CAC scores in patients with stable CVD for risk prediction, similar to the primary prevention setting, and computed tomography (CT) imaging costs and radiation exposure will not weigh up to the added prognostic value of CAC scores. However, in this patient population of patients with established CVD, CAC scores will often be available, with regard to several diagnostic indications for CT imaging. Furthermore, **chapter 5** showed that a model with presence or absence of CAC instead of continuous scores performed similarly. As the required scan settings might not always be available to calculate the Agatston score correctly (according to Agatston guidelines[37]), it could be valuable to develop models with addition of CAC scores continuously as well as presence or absence.

In contrast to CAC scores, thoracic aorta, and mitral annular and aortic valve calcium scores did not improve performance of a model with traditional atherosclerotic risk factors for the prediction of MACE+ in patients with stable CVD (**chapter 5**), and should not be used for this

purpose. The absence of added prognostic value of extra-coronary artery calcium scores is in line with studies in patients without established CVD,[38,39] and raise the question whether extra-coronary calcium scores are valuable in other populations or in different settings, or that these scores are simply uninformative for cardiovascular risk prediction in the presence of CAC scores.

There is a high correlation between CAC and extra-coronary artery calcium scores[40-42], and calcification is often multifocal as was shown in **chapter 4**. Furthermore, TAC scores were related to the outcome MACE+, but after addition of CAC scores, this relation was no longer evident (**chapter 4**). CAC might be the best measure for (subclinical) atherosclerotic burden, and additional, similar measures of atherosclerosis are therefore unlikely to provide valuable additional prognostic information for the prediction of atherosclerotic events on top of traditional risk factors and CAC scores.

Part III Individualized risk prediction

Personalized medicine

Prognostication has become an indispensable tool for determining preventive cardiovascular treatment strategies in current clinical practice, especially in primary prevention. Risk prediction in secondary prevention is steadily gaining ground, and cardiovascular risk scores, such as the SMART risk score, will be integrated into secondary prevention guidelines.

Risk prediction will guide the focus from a disease-centered point of view, considering similar treatment of all patients with CVD based on the fact that they have the 'same disease', to a more individual, personalized approach. Personalized medicine has become the general endeavor in current medical practice, a growing trend that started already more than two decades ago.[43,44] By estimating cardiovascular risks based on the patient's characteristics, preventive treatment strategies from lifestyle to therapeutic interventions, are fitted to the individual patient. Estimating the risk of the combined endpoint MACE+ as well as the risk of cancer, extends the focus beyond the scope of usual care for patients with stable CVD. This extension is a step further towards personalized medicine, by providing the best representation of an individual's prognosis, taking multiple aspects into account.

Furthermore, individual cardiovascular preventive treatment benefit can be estimated by means of established and accepted methods,[45-48] using hazard ratios from trials or meta-analyses.[15,46] This way, not only the objective clinical patient characteristics can be taken into account, but also patients' individual expectations towards preventive treatment. There is a high degree of variation within patients and in comparison to physicians in what is regarded meaningful treatment benefit, i.e. the number of estimated healthy life-years gained required to consider treatment. Patients consistently desired a higher treatment benefit than clinicians.[49] For statin treatment, the meaningful lifetime treatment benefit was 24 months (IQR 23 to 36 months) in clinicians (as users) and 42 months (IQR 12 to 42 months) in patients. The meaningful 10-year statin benefit was 12 months (IQR 10 to 12 months) in clinicians (prescribing) and 14 months (IQR 10 to 14 months) in patients.[49] These variations emphasize the importance of shared decision making, facilitated by discussing cardiovascular risks and potential treatment benefit.

Lung cancer risk prediction to lower thresholds for targeted diagnostics

High risks of cardiovascular disease could initiate therapeutic preventive strategies. Although this is not the case for cancer, high predicted risks of lung cancer could potentially lead to targeted diagnostics. Results from a recent lung-cancer screening trial (Nederlands–Leuvens Longkanker Screenings Onderzoek (NELSON) trial) showed that screening for lung cancer could reduce lung cancer mortality in men (cumulative rate ratio for death from lung cancer at 10 years of 0.76 (95%CI 0.61-0.94).[50] However, the all-cause mortality rate was similar (rate ratio 1.01

(95%CI 0.92-1.11),[50] suggesting the impact of screening is small on all-cause death as participants are also at high risk of dying from other smoking-related diseases. Furthermore, in order to select eligible former or current smokers, a total number of 606,409 persons were approached with a general questionnaire, whereas the total number of participants in the trial was 15,792, as the majority of the patients did not return the questionnaire, were not eligible, or did not provided written informed consent. The fact that the questionnaire concerned a trial might have led to fewer responses than in the case of a call for established screening. However, as opposed to current national screening programs for breast, cervical, and colorectal cancer, that invite people based on readily available information of age and sex, lung cancer screening eligibility requires smoking history. Inviting eligible patients is therefore challenging and a national screening program for lung cancer would be unfeasible. It could be hypothesized, though, that patients with stable CVD, who visit their specialist on a regular basis, and with a certain age and smoking history or the highest predicted 10-year risks for lung cancer as estimated by the model of **chapter 7**, could potentially benefit from regular CT-imaging of the chest. Furthermore, high predicted ten year risks of lung cancer could lead to lower thresholds for targeted diagnostics in patients with certain early symptoms, potentially contributing to earlier diagnosis and treatment of lung cancer.

Implementation of risk prediction in clinical practice

The currently available risk calculators for patients with stable cardiovascular disease to predict 10-year and lifetime risk of recurrent cardiovascular events, as well as treatment benefits, are available on www.U-Prevent.com. The models from **chapter 6** and **chapter 7** will also become available on this website to facilitate their use in clinical practice. The online calculators can be accessed by patients and physicians alike. The interpretation of the risk predictions might be best understood by patients with the corresponding explanation from a physician.

Current guidelines base treatment decisions on 10-year cardiovascular risk estimations. However, young patients with unfavorable risk factors profiles will have low 10-year risks, even though they will have the highest lifetime risks and will benefit the most from treatment with regard to healthy life-years gained. The transition from estimating 10-year risks, to measures such as lifetime risks and treatment benefits for determining preventive treatment strategies, assisted by the online accessible risk calculators, should be promoted.

Future research and perspectives

- Further studies should evaluate the efficacy of anti-inflammatory treatment in subgroups of patients with established CVD, such as in strata of patients with PAD and CeVD, in patients with inflammatory markers above and below a certain threshold level (for example CRP > 2 mg/L and ≤ 2 mg/L), or in patients with optimized lifestyle habits.

- The effects of quantifying cancer risks, from a 10-year as well as a lifetime perspective, and communicating these risks to patients with CVD on potential lifestyle improvements, and on subsequent reductions of cancer incidence should be evaluated in future studies.
- Future studies could aim to determine standardization and quantification of healthy lifestyle changes and their effects on recurrent cardiovascular events and cancer incidence.
- The added prognostic value of CAC scores for the prediction of MACE+ should be reevaluated in future studies in a study population with longer follow-up duration and more endpoints, as well as the added prognostic value of CAC for the outcome recurrent MACE specifically.
- Future studies should explore the impact of CAC scoring as addition to risk prediction models and the subsequent therapeutic consequences and the effect on cardiovascular morbidity and mortality. As the model with CAC scores or CAC presence/absence particularly reclassifies patients without an event to a lower risk, potentially leading to downgrading preventive treatment strategies, therapy harms and costs should also be taken into account in such a study.
- Since the combined endpoint MACE+ is not usually reported as outcome in trials or meta-analyses, future evaluations should deliberate whether the relative treatment effect measures reported for recurrent MACE specifically or cardiovascular interventions separately could be used for this combined endpoint as well, in order to allow for estimations of treatment benefit regarding MACE+ by the extended SMART risk score.
- Shared decision making based on lifetime cardiovascular risks and treatment benefits expressed in healthy life-years gained, rather than 10-year risks, should be promoted.

Concluding remark

Today, we are facing the challenge of a growing number of patients with established CVD, and their risks of recurrent cardiovascular events well as risk of cancer. The results of this thesis provide insight in systemic low-grade inflammation, cardiovascular calcification, and individualized risk prediction in patients with stable CVD, in order to maximize the number of healthy life-years for these patients, and to reduce the global burden of disease.

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Appendices

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Summary

The number of patients with established cardiovascular disease (CVD) in a chronic phase is growing as a consequence of several factors, including the increased survival of patients with an acute manifestation of CVD, population growth and ageing, and lifestyle habits such as sedentary behavior and obesity. Patients with established CVD are at risk of recurrent cardiovascular events as well as at risk of cancer, due to similar risk factors. Preventing second cardiovascular events and incident cancer in patients with established CVD is needed from a patient's, as well as an economic perspective.

In this thesis, risk factors and risk prediction beyond the scope of current usual care were investigated in patients with stable CVD, by means of three main topics: Systemic low-grade inflammation, cardiovascular calcification, and individualized risk prediction.

Part I Systemic low-grade inflammation

Shared risk factors for CVD and cancer include smoking, diabetes mellitus, and obesity. Inflammation is considered one of the major common underlying pathophysiological pathways leading from these risk factors to CVD and cancer. In **chapter 2** it was shown that systemic low-grade inflammation, measured by high sensitive C-reactive protein (CRP) ≤10 mg/L, is a risk factor for recurrent cardiovascular events as well as incident cancer in patients with stable CVD. The relation between CRP and lung cancer was most evident, with a HR of 1.16 (95% CI 1.10-1.22) per 1 mg/L higher CRP, corresponding to previous observational studies in patients without CVD and the CANTOS trial. These results suggest that patients with established CVD and high inflammatory levels might benefit from lowering inflammation, with regard to the reduction of recurrent cardiovascular risk as well as (lung) cancer risk.

Chapter 3 showed that lifestyle improvements (smoking cessation, weight loss, and increase of physical activity) reduced low-grade inflammation in patients with established CVD. These relations may indicate that part of the effect of lifestyle improvements on the reduction of cardiovascular risk might be through lowering inflammation. Targeting lifestyle habits should be the first preventive strategy to reduce low-grade inflammation. However, **chapter 3** also found that only few patients with stable CVD improved lifestyle habits, despite not achieving guideline recommended goals, and more emphasis and assistance on lifestyle improvements in clinical practice could be beneficial for attainment of lifestyle goals.

Part II Cardiovascular calcification

Cardiovascular calcification is a complicated and multifaceted process. **Chapter 4** showed that cardiovascular calcification is generally multifocal (in 75% of the patients) in patients with established CVD, although only 4% of the patients had calcification in thoracic aorta, mitral annulus, aortic valve, and coronary arteries simultaneously. Furthermore, **chapter 4** showed that associations between atherosclerotic risk factors and calcification at the different locations varied, stressing the divergence in pathophysiological pathways. Although the causal effect of cardiovascular calcification on (recurrent) cardiovascular events is unclear, a relation was observed between coronary artery calcium (CAC) scores and a combined endpoint of recurrent major cardiovascular events and cardiovascular interventions (MACE+), adjusted for traditional atherosclerotic risk factors and calcium scores of thoracic aorta and heart valves (HR 1.39; 95%CI 1.15-1.68).

Chapter 5 continued from this last finding and the added prognostic value of cardiovascular calcification scores for the prediction of the combined endpoint MACE+ was investigated in this chapter. A model with addition of CAC scores to traditional atherosclerotic risk factors improved risk predictions for patients with stable CVD. Similar to the primary prevention, it could be recommended to implement CAC scores in cardiovascular risk prediction for patients with established CVD and available CAC scores, to assist in the determination of preventive treatment strategies. Calcium scores of thoracic aorta and heart valves did not improve risk prediction. As the number of events was limited (N=77), these results are preliminary and should be confirmed in future studies.

Part III Individualized risk prediction

In order to successfully prevent a second cardiovascular event in patients with established CVD, preventive treatment strategies should be personalized to fit each individual patient, based on cardiovascular risk and treatment benefit predictions. As addition to the SMART risk score, in **chapter 6** the extended SMART risk score was developed. The extended SMART risk score predicts the combined risk of major recurrent cardiovascular events and cardiovascular interventions (MACE+), as cardiovascular interventions are clinically relevant from a patient's as well as an economic point of view. The extended SMART risk score was developed in the UCC-SMART cohort (N=8,421) and externally validated in REACH Western Europe (N=14,528) and REACH North America (N=19,495). It was shown that the combined endpoint MACE+ can be estimated in patients with established coronary heart disease or cerebrovascular disease. However, cardiovascular interventions in patients with peripheral artery disease could not be predicted reliably with the developed risk model.

Similar to recurrent cardiovascular risk, the risk of cancer varies in individual patients with established CVD. In **chapter 7**, risk scores with easily clinically available predictors were developed in the UCC-SMART cohort and externally validated in the CANTOS trial study population for the prediction of total cancer, colorectal cancer, and lung cancer in patients with stable CVD. The models allow for 10-year risk predictions, as well as lifetime risk predictions. These lifetime risk predictions might provide a more accurate representation of an individual's actual risk, especially for young patients with an unfavorable risk factor profile. Estimating personalized probabilities could aid in an individual patient's and clinicians' understanding of cancer risk, emphasize lifestyle improvements such as smoking cessation, and potentially lower thresholds for targeted diagnostics in individual patients.

Samenvatting
(voor niet ingewijden)

Deze samenvatting beschrijft in het kort de resultaten en relevantie van de studies in dit proefschrift. Het overkoepelende thema van dit proefschrift is de zorg voor patiënten in een stabiele fase van hart- en vaatziekte.

Het aantal patiënten met hart- en vaatziekte in een chronische fase neemt toe als gevolg van een verbeterde overlevingskans na acuut hart- en vaatlijden, bevolkingsgroei, vergrijzing en leefstijlfactoren zoals verminderde beweging en overgewicht. Patiënten met hart- en vaatziekte hebben zowel een verhoogd risico op het krijgen van een tweede uiting van hart- en vaatziekte, als op het krijgen van kanker door gemeenschappelijke risicofactoren zoals roken en overgewicht. Het voorkómen van een nieuwe uiting van hart- en vaatziekte en het voorkómen van kanker is belangrijk vanuit het perspectief van de patiënt en vanuit een economisch perspectief.

In dit proefschrift wordt de focus gelegd op de zorg voor patiënten in een stabiele fase van hart- en vaatziekte buiten het kader van de reguliere zorg, aan de hand van drie thema's: Laaggradige ontsteking, verkalkingen van hartkleppen en vaten in en rond het hart, en risicovoorspellingen voor de individuele patiënt.

Deel I Laaggradige ontsteking

Laaggradige ontsteking, niet te verwarren met een ontsteking bij een acute infectie, is een chronische staat van ontsteking. Leefstijlfactoren zoals roken en overgewicht verergeren deze chronische ontsteking. De mate van laaggradige ontsteking kan in het bloed gemeten worden met de biologische marker C-reactive Protein (CRP); hoe hoger het CRP gehalte, hoe meer ontsteking. Hart- en vaatziekte en kanker hebben gemeenschappelijke risicofactoren, waaronder roken, diabetes mellitus en overgewicht. Laaggradige ontsteking wordt beschouwd als één van de belangrijkste onderliggende mechanismen tussen deze gemeenschappelijke risicofactoren en het ontstaan en de progressie van hart- en vaatziekte en kanker. **Hoofdstuk 2** toont aan dat een hogere mate van ontsteking bij patiënten met vaatziekte een hoger risico geeft op acute hart- en vaatziekte en ook het krijgen van kanker. Met name het risico op longkanker is verhoogd bij een hogere mate van ontsteking. Deze resultaten komen overeen met eerdere studies en geven aan dat patiënten in een stabiele fase van hart- en vaatziekte met verhoogde ontstekingswaarden baat kunnen hebben bij het verlagen van ontsteking. Hierdoor kunnen nieuwe hart- en vaatziekten worden voorkomen, en wordt daarbij mogelijk ook het risico op (long)kanker gereduceerd.

Eerdere studies hebben laten zien dat medicijnen chronische ontsteking kunnen verlagen bij patiënten met hart- en vaatziekte. **Hoofdstuk 3** laat zien dat leefstijlverbeteringen (stoppen met roken, gewichtsverlies en meer lichamelijke beweging) de mate van ontsteking ook verlagen. Deze resultaten impliceren dat behandelstrategieën voor het verlagen van ontsteking ter preventie van hart- en vaatziekte (en kanker), zich als eerste zouden moeten richten op leefstijlfactoren. Echter, **hoofdstuk 3** laat ook zien dat slechts een klein deel van de patiënten met bestaande hart- en vaatziekte hun leefstijl verbetert, ondanks het niet behalen van geadviseerde leefstijldoelen. Meer nadruk en begeleiding bij het behalen van leefstijldoelen in de klinische praktijk zou dit mogelijk kunnen verbeteren.

Deel II Verkalkingen van hartkleppen en vaten in en rond het hart

De mate van slagader- en hartklepverkalking kan worden gekwantificeerd in een kalkscore die berekend wordt op basis van een CT-scan. Hoe hoger de score, hoe meer kalk er aanwezig is. Met behulp van deze kalkscore is in **hoofdstuk 4** gekeken naar oorzaken van verkalkingen in de kransslagers, aorta (grote lichaamsslagader) en hartkleppen in mensen met hart- en vaatziekte. Het proces van verkalking is namelijk gecompliceerd en veelzijdig en is nog niet geheel ontrafeld. Hoewel verkalking vaak wordt beschouwd als een gegeneraliseerd proces, zijn er ook verschillen in lokale mechanismen, waardoor de ene patiënt kalk krijgt in de kransslagaders en de andere patiënt op de hartkleppen. **Hoofdstuk 4** laat zien dat de meerderheid van de patiënten (75%), verkalkingen heeft op meerdere plekken. Echter, slechts 4% van de patiënten had verkalkingen op hartkleppen, kransslagaders en aorta (grote lichaamsslagader) tegelijk. Ook laat **hoofdstuk 4** zien dat de associatie tussen risicofactoren voor hart- en vaatziekte zoals roken en diabetes, en de ernst van verkalkingen op de verschillende locaties varieert. Deze observaties benadrukken dat er verschillen zijn in mechanismen van verkalking.

Naast het onderzoeken van de oorzaken van kalk, kan de kalkscore ook worden gebruikt om het risico op nieuwe manifestaties van hart- en vaatziekte te voorspellen voor een individuele patiënt met bestaande hart- en vaatziekte. Het is daarbij de vraag of de kalkscore extra informatie toevoegt aan reeds bekende factoren zoals leeftijd, geslacht, roken, cholesterolwaarden en diabetes. Dit is onderzocht in **hoofdstuk 5**. Een standaard voorspelmodel met gebruikelijke factoren (zoals roken, diabetes, leeftijd, etc) is vergeleken met voorspelmodellen met toevoeging van kalkscores van de kransslagaders, aorta en hartkleppen, om het risico op hart- en vaatziekte te voorspellen. Het toevoegen van de kalkscore van de kransslagaders verbetert de risicovoorspellingen. Toevoeging van kalkscores van de aorta en de hartkleppen levert geen verbetering op. De resultaten van **hoofdstuk 5** zullen in toekomstig onderzoek in meer patiënten die langer gevolgd zijn, moeten worden bevestigd.

Aangezien kalkscores van de kransslagers risicovoorspellingen verbeteren, zouden deze kalkscores gebruikt kunnen worden om het risico voor een individuele patiënt met bestaande hart- en vaatziekte zo precies mogelijk te schatten. In de klinische praktijk zal dit alleen geadviseerd worden indien een kalkscore al beschikbaar is. Het is niet waarschijnlijk dat de voordelen van het bepalen van de kalkscore door middel van een CT-scan opwegen tegen de stralingsbelasting voor de patiënt en de kosten.

Deel III Risico voorspellingen voor de individuele patiënt

Accurate risicovoorspellingen zijn onmisbaar voor het bepalen van een passende preventieve behandelstrategie voor een individuele patiënt en om deze behandelkeuzes samen met de patiënt te maken. Een veelgebruikte risicoscore voor patiënten met bestaande hart- en vaatziekte, de SMART risicoscore, voorspelt het risico op hart- en vaatziekte en het effect van behandelingen op dat risico. In **hoofdstuk 6** is een model ontwikkeld, als toevoeging aan de SMART risicoscore, voor het gecombineerd voorspellen van hart- en vaatziekte en vaatinterventies, zoals open hart operaties, dotterbehandelingen en amputaties.

Net als het risico op hart- en vaatziekte, varieert ook het risico op kanker in individuele patiënten met hart- en vaatziekte. In **hoofdstuk 7** zijn voorspelmodellen ontwikkeld om het risico op longkanker, darmkanker en totaal kanker te schatten. De modellen werden gemaakt om niet alleen het 10-jaars risico te schatten, maar ook het levenslange risico op kanker. Deze schattingen van het levenslange risico geven een relevantere risicoschatting van een individu, vooral bij jonge patiënten met een ongunstig risicoprofiel. Het voorspellen van deze risico's zouden kunnen helpen bij het benadrukken van het belang van een gezonde leefstijl, zoals het stoppen met roken. Ook zou er bij patiënten met hoge voorspelde risico's mogelijk eerder diagnostisch onderzoek worden ingezet, waardoor kanker in een vroeger stadium kan worden opgespoord.

Concluderend, bij patiënten met hart- en vaatziekte is chronische ontsteking een risicofactor voor nieuwe hart- en vaatziekte en kanker, en dragen leefstijlverbeteringen bij aan de verlaging van ontsteking. Om een zo goed mogelijke inschatting te geven van risico's voor een individuele patiënt met hart- en vaatziekte in de klinische praktijk, kunnen kalkscores van de kransslagaders worden gebruikt en kan het risico op hart- en vaatziekte met vaatinterventies en het risico op kanker worden voorspeld.

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Cilie

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Curriculum vitae

Cilie was born on the 9th of March 1991 in Haarlem, the Netherlands. After graduating cum laude from the Christelijk Lyceum Zeist in Zeist in 2009, she studied Medicine at Utrecht University. During her studies she worked at the research and development division of the cardiology department at the "Antonius Ziekenhuis" in Nieuwegein, raising her scientific interest. She completed her senior internship at the internal department at the "Ziekenhuis Gelderse Vallei" and continued working there after obtaining her medical degree in 2016. In 2017 she started her PhD project on inflammation, calcification, and risk prediction in patients with stable cardiovascular disease at the University Medical Center Utrecht, under the supervision of prof. dr. F.L.J. Visseren (Department of Vascular Medicine) and prof. dr. Y. van der Graaf (Julius Center for Health Sciences and Primary Care). She combined her PhD research with a Postgraduate Masters in Clinical Epidemiology at Utrecht University and obtained this degree in July 2019. In August 2020 she started working as a resident at a psychiatric outpatient clinic specializing in bipolar disorders.