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Associations between cardiovascular risk factors, inflammation, and progression of carotid atherosclerosis among smokers

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Abstract

Introduction:

The high risk of cardiovascular events in smokers requires adequate control of other cardiovascular risk factors (CVRFs) to curtail atherosclerosis progression. However, it is unclear which CVRFs have the most influence on atherosclerosis progression in smokers.

Methods:

In 260 smokers aged 40-70 included in a smoking cessation trial, we analyzed the association between traditional CVRFs, high-sensitivity C reactive protein (hs-CRP), smoking cessation and 3-year progression of carotid intima-media thickness (CIMT, assessed by repeated ultrasound measurements) in a longitudinal multivariate model.

Results:

Participants (mean age 52 years, 47% women) had a mean smoking duration of 32 years with a median daily consumption of 20 cigarettes. Baseline CIMT was 1185µm (95%CI:1082-1287) and increased by 93µm (95%CI:25-161) and 108µm (95%CI:33-183) after one and three years, respectively. Age, male sex, daily cigarette consumption, systolic blood pressure (SBP), but neither LDL-cholesterol nor hs-CRP, were independently associated with baseline CIMT (all $p \leq 0.05$). Baseline SBP, but neither LDL-cholesterol nor hs-CRP, was associated with 3-year atherosclerosis progression ($p=0.01$ at 3 years). The higher the SBP at baseline, the steeper was the CIMT increase over 3-year follow-up. We found an increase of 26µm per each 10mmHg raise in SBP at one year and an increase of 39µm per each 10mmHg raise in SBP at three years. Due to insufficient statistical power, we could not exclude an effect of smoking abstinence on CIMT progression.

Conclusion:

Control of blood pressure may be an important factor to limit atherosclerosis progression in smokers, besides support for smoking cessation.

Implications

Among 260 smokers aged 40-70 years with a mean smoking duration of 32 years, baseline systolic blood pressure was associated with atherosclerosis progression over 3 years, as measured by carotid intima-media thickness (CIMT, p=0.01 at 3 years), independently of smoking variables and other cardiovascular risk factors. The higher the SBP at baseline, the steeper was the CIMT increase over 3-year follow-up. Our findings emphasize the importance of focusing not only on smoking cessation among smokers, but to simultaneously control other cardiovascular risk factors, particularly blood pressure, in order to prevent future cardiovascular disease.

Key Words: smoking; carotid artery disease; risk factors; ultrasonography; intima-media thickness; C-reactive protein.

Introduction

Smoking increases the risk of cardiovascular diseases (CVD) by enhancing atherosclerosis process[1–4] and shifting the balance of homeostasis towards thrombus formation[4,5]. Several studies have shown an association between smoking and carotid intima-media thickness (CIMT)[2,6–8], atherosclerotic disease[9] and CIMT progression[10]. Besides smoking, traditional cardiovascular risk factors (CVRFs), such as age, male sex, hypertension, hypercholesterolemia, diabetes and obesity, have also been associated with the atherosclerosis progression[7,8,11–14]. Most previous studies examining the role of CVRFs with atherosclerosis development were conducted in mixed populations consisting of smokers, former smokers and non-smokers[15,16], or often focused on young populations[10]. However, there are few available data regarding smokers only, and not all smokers are at similar risk for CVD, because intensity of exposure influences atherosclerosis development[17]. Thus, it remains unclear which CVRFs have the most influence on atherosclerosis progression in smokers. Therefore, we examined whether traditional CVRFs, subclinical inflammation and smoking cessation were associated with atherosclerosis progression over a 3-year follow-up in smokers included in a smoking cessation trial[18].

Methods

We prospectively studied smokers aged 40-70 years without preexisting CVD from the CAROtid plaque Screening trial on Smoking cessation (CAROSS), a randomized controlled trial[18,19] designed to assess the benefit of carotid atherosclerotic disease screening along with advice to quit smoking. Details of the methods have been previously reported[19]. Current smokers of ≥10 cigarettes/day motivated to quit were recruited through newspaper advertisements. 536 participants aged 40-70, without preexisting CVD, were included and randomized into carotid atherosclerotic disease screening by ultrasound (intervention group) vs. no ultrasound (control group) in addition to smoking cessation counseling for both groups. We examined 266 smokers of the intervention group screened by ultrasound for atherosclerosis at baseline and with ultrasound data at 1 and 3-year follow-up. Six participants were excluded due to missing laboratory data or non-measurable carotid plaques. Thus data on 260 participants were available for the current analysis.

Cardiovascular risk factors

As previously described[19], we collected demographics, physical activity, medical and smoking history, and medication use at baseline. We measured weight, height, blood pressure (BP), and fasting plasma levels of lipids and glucose using standardized techniques. BP was measured using a standard sphygmomanometer after a 10-minute rest in seated position[20]. The right-arm BP was assessed at three 1-minute-intervals, the two final BP averaged to reduce variability. Hypertension was defined as systolic blood pressure (SBP) ≥140mmHg and/or diastolic blood pressure (DBP) ≥90mmHg; or SBP ≥130mmHg and/or DBP ≥80mmHg for participants with diabetes, and/or on anti-hypertensive treatment[20]. Hypercholesterolemia was defined as LDL-cholesterol over

target value recommended by ATP-III guidelines[21] or on lipid-lowering medication. Diabetes was defined as fasting plasma glucose ≥7.0mmol/l or on anti-diabetic treatment[22]. High-sensitivity C-reactive protein (hs-CRP) was measured by immunonephelemetric assay (Siemens Healthcare Diagnostics, Marburg, Germany). Duration of smoking before inclusion in the study was calculated by deducting self-reported periods of abstinence. We defined continuous abstinence during follow-up as complete smoking cessation between baseline and follow-up visits, with repeated biochemical validation of smoking status by exhaled carbon monoxide and plasma cotinine measurements[18]. Participants who were smoking at the time of 1-year or 3-year follow-up visits were defined as 1-year or 3-year relapse. The local Ethical Committee approved the study and all participants gave written informed consent.

B-mode ultrasound examination

Participants had B-mode carotid ultrasound screening for atherosclerotic lesions (System 5; Vingmed, General Electric), performed by a trained observer blinded to clinical information at baseline, 1 year, and 3 years. The intraobserver variability showed 95% agreement between the first and second reading among 20 randomly selected subjects[18]. We measured maximal CIMT of the right and left carotid artery bifurcation and averaged both to obtain mean maximal CIMT[23]. The current resolution of 1 pixel corresponds to a surface of 54x54µm. Measurements were conducted according to the recommendations of the US Task Force on noninvasive atherosclerosis measurement[24] and similar to the Rotterdam Study[18,25].

Statistical analysis

A multivariate longitudinal model was used to assess the association between baseline CVRFs and CIMT and its progression over 3-year follow-up. Longitudinal models are methods of choice for analyzing data with repeated measures for each participant[26,27] and allow to estimate an adjusted mean outcome trajectory, taking into account the inter-individual variability[28,29]. In addition, they can deal with unbalanced data due to drop-out and missing values. To account for their additive effect, all baseline CVRFs were entered in the model, smoking abstinence as a time-varying covariable. CVRFs with skewed distributions (number of cigarettes per day; hs-CRP) were log-transformed. Three smoking abstinence trajectories were considered, corresponding to different levels of smoking exposure, depending on abstinence status at each follow-up visit: "one-year relapse", "one-year continuous abstinence and three-year relapse", and "three-year continuous abstinence". We also tested for possible interactions between smoking abstinence and SBP and LDL-cholesterol respectively. For this analysis, smoking abstinence was dichotomized in 'at least one year continuous abstinence' and 'relapse' for better legibility of the results.

Statistical analyses were performed using R software package (version 2.15.1, GNU Project, University of Auckland, New Zealand).

Results

Baseline characteristics of the 260 participants are described in Table 1. Participants had a mean duration of 32 years of smoking and a large prevalence of CVRFs. Forty-one participants could not be assessed for CIMT at 1-year and 83 participants at 3-year follow-up. There was no significant difference between baseline characteristics of participants without CIMT measurements at 3-year follow-up (n=83) compared to those with follow-up (n=177), except for a borderline statistically significant lower SBP (p=0.05).

Estimated CIMT progression was 93μm after 1-year and 108μm after 3-year follow-up, respectively (Table 2, Appendix Figure). A dose-response relationship was found between number of cigarettes per day at baseline and baseline CIMT (p=0.03). Male sex, age and SBP were also significantly associated with baseline CIMT (all $p \leq 0.05$) (Table 2). For instance, baseline CIMT is estimated to be 50.8μm larger per each 10mmHg increase in baseline SBP. In both sexes, 1-year and 3-year CIMT progression was similar (p for interaction= 0.96 and p=0.97, respectively), but baseline CIMT differed (p= 0.04) (Table 2, Appendix Figure).

Baseline SBP was also associated with CIMT progression over 3-year follow-up, showing borderline significance at 1-year (p=0.06) and significance at 3-year follow-up (p=0.01). CIMT increase at one year was estimated to be 26μm larger per each 10mmHg increase in SBP (Table 2) and 39μm larger per each 10mmHg increase in SBP at three years compared to baseline (Table 2). Thus, the higher the SBP at baseline, the steeper was the CIMT increase over 3-year follow-up for both female and male participants (Appendix Figure). We found no significant associations of gender with the progression of CIMT

(p=0.96 at 1-year, p=0.97 at 3-year follow-up). In a sensitivity analysis, we added the covariables of medication for hypertension, hypercholesterolemia and hyperglycemia at baseline in our longitudinal model; none of the three covariables showed a significant association with CIMT, but SBP remained significantly associated with baseline CIMT (p=0.05) and after 3 years (p=0.02), comparable to results in Table 2.

An attenuation in CIMT progression was observed after one and three years of continuous abstinence with respect to the reference group (1-year relapse), although not reaching statistical significance (Table 2). We did not find statistically significant interactions between continuous smoking abstinence and SBP or LDL-cholesterol after 3-year follow-up (p=0.23 and p=0.15 respectively).

Discussion

Among smokers, we found that BP was associated with atherosclerosis progression over 3-year follow-up beyond smoking variables and other CVRFs. By contrast, we did not find an association between subclinical inflammation measured by hs-CRP, or other CVRFs and atherosclerosis progression.

Few data exist about the association of CVRFs and carotid atherosclerosis progression in smokers. Our results are consistent with the San Daniele Project that found that age, SBP and smoking were associated with carotid plaques in participants >39 years both at baseline[7] and at 12-year follow-up[8]. However, smoking duration was not reported in this study and definitive or transient smoking cessation was likely not taken into account in these analyses. Johnson et al.[10] found an association between SBP and CIMT progression as well as the number of cigarettes smoked per day. However, this study focused on young adults (n=336, mean age 32 years) and neither the association

between LDL-cholesterol and CIMT progression, nor the prevalence of hypertension was reported. Similar to our study, a randomized controlled trial of smoking cessation among 795 smokers with a 3-year follow-up[11] found that antihypertensive medication use was associated with increase of CIMT (i.e. an indirect indication that BP was associated with CIMT). However, participants with uncontrolled hypertension (BP>160/100mmHg) were excluded and the proportion of patients with hypertension was not mentioned. Similar to our study, smoking status (cessation or continuation) during 3-year follow-up did not predict CIMT progression beyond CVRFs.

Hs-CRP was not associated with CIMT in our multivariate analysis, neither at baseline, nor after 1 or 3-year follow-up. This finding is consistent with the results by Lorenz et al.[16], which demonstrated that the association between hs-CRP and baseline CIMT disappeared after controlling for conventional CVRFs. The absence of an association between hs-CRP and carotid plaques in multivariate analysis was also shown by Molino-Lova et al.[15]. However, this study showed a significant association between higher hs-CRP levels and the 3-year incidence of carotid plaques, hypothesizing that hs-CRP represents the overall inflammatory burden or the early stage of vessel inflammation. Both of the above mentioned studies included smokers and non-smokers without specifying their respective repartition and hs-CRP levels in smokers were not compared to those in non-smokers. Our study is the first to focus on smokers only and the absence of an association between hs-CRP and CIMT is generally consistent with increasing evidence that CRP is a marker but not an independent risk factor of CVD, as shown for example in Mendelian randomization studies[30].

In our results, LDL-cholesterol had a pattern of association (albeit not statistically significant) with baseline CIMT, but not with CIMT progression. A similar finding by Molino-Lova et al.[15] showed no significant association between baseline LDL-cholesterol and the appearance of new carotid plaques over 3 years. Johnson et al.[11] described an association between 3-year changes in LDL-cholesterol and CIMT progression, but did not examine the association of baseline LDL-cholesterol and CIMT progression. A longer follow-up period might be needed to detect a possible association between LDL-cholesterol and CIMT progression, although a 3-year follow-up revealed an association with BP.

Our study had several limitations. By design, we included only smokers motivated to quit and these persons might have healthier behaviors than average smokers. However, the prevalence of CVRFs was high at baseline. Calculation of smoking abstinence relied on self-reported abstinence, confirmed by measurement of exhaled carbon monoxide and serum cotinine at follow- up visits[18]. However, we could not exclude smoking exposure between visits. Although CIMT progression was slower in participants continuously abstinent compared to those relapsing after 1 year, these results did not reach statistical significance, likely because our sample size was relatively small. Our study may have been underpowered to demonstrate weak associations of other risk factors or continued smoking cessation on CIMT progression. As we did not measure genetic factors, we could not assess their effect on CIMT.

Among strengths, we were able to analyze data of a 3-year follow-up, as well as characterize participants accurately according to smoking exposure, expressed as number of cigarettes per day, years of smoking and pack-years, as well as smoking status during the study follow-up, compared to previous studies[3,8,9]. Although the

sample was not large enough to assess small effects of exposures, particularly that of smoking discontinuation, our study is one of the largest to assess the impact of CVRFs among well-characterized smokers.

In conclusion, several CVRFs in smokers are associated with carotid atherosclerosis in cross-sectional analyses, but BP was the strongest predictor of atherosclerosis progression among smokers. Hs-CRP is not associated with baseline or carotid atherosclerosis progression. Our findings emphasize the importance of focusing not only on smoking cessation among smokers, but to simultaneously control other CVRFs, particularly BP, in order to prevent future CVD.

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Declaration of Conflicting Interests

None declared.

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Table 1. Characteristics of study participants (n=260)

Demographics	
Age, years, mean (SD)	51.6 (7.6)
Women, % (nb)	46.5 (121)
Smoking history	
Number of cigarettes per day, median [IQR]	20 [20, 30]
Duration of tobacco smoking[a], years, mean (SD)	32.1 (8.5)
Number of pack-years, mean (SD)	39.1 (19.7)
Cardiovascular risk factors	
Systolic blood pressure, mmHg, mean (SD)	123.7 (15.8)
Hypertension[b], % (nb)	32.7 (85)
HDL cholesterol, mmol/l, mean (SD)	1.4 (0.41)
Calculated LDL cholesterol[c], mmol/l, mean (SD)	3.7 (0.97)
Hypercholesterolemia[d], % (nb)	47.3 (123)
Fasting plasma glucose, mmol/l, mean (SD)	4.9 (0.87)
Diabetes[e], % (nb)	3.8 (10)
hs-CRP, mg/dl, median [IQR]	1.35 [0.69, 3.01]
Lifestyle	
Alcohol, standard units per week[f], median [IQR]	6 [3, 15]

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; IQR, interquartile range; LDL, low-density lipoprotein.

[a] Periods of self-reported interruption deducted

[b] Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥ 90 mmHg; or systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥80 mmHg for participants with diabetes; or on anti-hypertensive treatment [20]

[c] LDL cholesterol was calculated according to the Friedewald equation, with the exclusion of 1participant with fasting triglycerides above 4.5 mmol/l (400 mg/dl) [31]

[d] Hypercholesterolemia was defined as LDL cholesterol over target value recommended by NCEP ATP-III guidelines or on lipid-lowering medication [32]

[e] Diabetes was defined as fasting plasma glucose ≥7.0 mmol/l or on anti-diabetic treatment [22]

[e] Cardio- and cerebrovascular disease of first-degree relatives (men<55years, women<65years) [21]

[f] 21 missing values for self-reported alcohol consumption.

Table 2:
Multivariate longitudinal analysis of the effect of cardiovascular risk factors on carotid intima-media thickness at baseline and on its progression over 3 years of follow-up.

	Baseline (n=260)				1-year follow-up (n=219)				3-year follow-up (n=177)			
	Estimate[b]	P value	95% CI		Estimate[b]	P value	95% CI		Estimate[b]	P value	95% CI	
Mean CIMT-max at baseline and progression, µm	1184.6		1082.1	1287.0	93.0		25.3	160.6	108.2		33.2	183.2
Male sex	157.6	0.04	7.1	308.2	2.3	0.96	-90.6	95.1	2.2	0.97	-99.5	103.8
Age (per 10 years)	429.3	<0.001	291.6	567	36.3	0.40	-47.6	120.2	-9.4	0.83	-98.1	79.2
Log number of cigarettes per day at baseline	196.1	0.03	20.6	371.6	-9.8	0.86	-119.6	100.1	-96.5	0.11	-214.9	21.9
Years of smoking	4.2	0.49	-7.7	16.1	-3.5	0.35	-10.8	3.8	-5.0	0.20	-12.6	2.6
SBP at baseline (per 10 mmHg)	50.8	0.03	5.9	95.8	26.1	0.06	-1.5	53.7	39.0	0.01	9.2	68.7
LDL cholesterol at baseline, mmol/l	51.4	0.14	-16.9	119.7	27.5	0.21	-15.2	70.3	6.9	0.78	-40.4	54.1
HDL cholesterol at baseline, mmol/l	-123.0	0.19	-308.5	62.6	-43.4	0.48	-164.7	77.9	74.5	0.27	-59.0	208.1
Fasting plasma glucose at baseline, mmol/l	-26.2	0.52	-105.6	53.2	11.4	0.68	-42.8	65.7	-68.5	0.03	-128.6	-8.4
Log hs-CRP at baseline, mg/dl	-35.5	0.30	-103.2	32.1	-14.1	0.51	-56.1	28.0	25.2	0.29	-21.2	71.5
Smoking abstinence[a]												
1-year relapse (reference category)	n/a				ref.				ref.			
1-year abstinent / 3-year relapse	n/a				-24.1	0.61	-115.7	67.6	-67.3	0.47	-251.7	117.2
3-year abstinent	n/a				-24.1	0.61	-115.7	67.6	-16.0	0.76	-119.2	87.2

Abbreviations: CIMT-max, carotid intima-media thickness; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

[a] Numbers of participants in categories of smoking abstinence: 1-year relapse: n=208; 1-year abstinent/3-year relapse: n=13; 3-year continuously abstinent: n=39

[b] Estimates in the first line of the table indicate the actual mean thickness of CIMT (in micrometers), determined by all cardiovascular risk factors mentioned in the table (baseline CIMT=1185µm).

Estimates in the other lines of the table (except line 1) correspond to change in CIMT (in micrometer) associated with a one-unit change of a risk factor at baseline, adjusted to all covariates mentioned in the table. Estimates at 1 year refer to change in CIMT between baseline and 1 year, and estimates at 3 years refer to change in CIMT between baseline and 3 years.