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Relation of Smoking Status to a Panel of Inflammatory Markers: The Framingham Offspring

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Abstract

Aims—We sought to investigate the hypothesis that smoking is accompanied by systemic inflammation.

Methods and Results—We examined the relation of smoking to 11 systemic inflammatory markers in Framingham Study participants (n=2944, mean age 60 years, 55% women, 12% ethnic minorities) examined from 1998–2001. The cohort was divided into never (n=1149), former (n=1424), and current smokers with last cigarette >6 hours (n=134) or ≤6 hours (n=237) prior to phlebotomy. In multivariable-adjusted models there were significant overall between-smoking group differences (defined as $p<0.0045$ to account for multiple testing) for every inflammatory marker tested, except for serum CD40 ligand (CD40L), myeloperoxidase (MPO) and tumor necrosis factor receptor-2 (TNFR2). With multivariable-adjustment, pair-wise comparisons with never smokers revealed that former smokers had significantly lower concentrations of plasma CD40L ($p<0.0001$) and higher concentrations of C-reactive protein ($p=0.002$).

Conclusions—As opposed to never smokers, those with acute cigarette smoke exposure (≤6 hours) had significantly higher concentrations of all markers ($p<0.0001$) except serum CD40L, MPO, and TNFR2; plasma CD40L were significantly lower. Compared with never smokers, cigarette smokers have significantly elevated concentrations of most circulating inflammatory markers, consistent with the hypothesis that smoking is associated with a systemic inflammatory state.

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Keywords

smoking; inflammation; cardiovascular disease; epidemiology

Introduction

Cigarette smoking confers a higher risk for peripheral arterial disease, myocardial infarction, heart failure,[1] and stroke,[2] as well as for lung, pancreatic, renal, cervical, and gastric cancers.[3] Whereas efforts have been made to reduce cigarette smoking in public areas both in Europe and North America, 17.5%– 45% of adults in the European Union and United States continue to smoke.[4] Because those in lower socioeconomic strata tend to smoke more, a study of adult men in Europe and the United States revealed that smoking accounted for almost half of the mortality in persons in the lowest socio-economic stratum.[5,6] Additionally, a study of adolescents in ten European countries reported up to 20% of boys and 24.7% of girls smoke daily, implying that smoking-related health complications will persist in the next 20–30 years.[7] The World Health Organization estimates that of the 1.1 billion smokers worldwide, 800 million (73%) are in developing nations,[8] thereby placing a disproportionate burden on those health-care systems. Globally, cigarette smoking remains a major public health concern and a premier modifiable risk factor for cardiovascular disease.

We and others have noted that systemic markers of inflammation, such as C-reactive protein (CRP),[9–14] fibrinogen,[10,12,14] interleukin-6 (IL-6)[9,11] soluble intercellular adhesion molecule-1 (ICAM-1),[15] and monocyte chemoattractant protein-1 (MCP-1)[16] are elevated in smokers but there is little data on other novel markers of inflammation in smokers such as, myeloperoxidase (MPO) and tumor necrosis factor receptor 2 (TNFR2). Whereas previous studies have examined inflammatory markers in smokers, they have been limited by small sample sizes,[9,17] hospital-based samples,[17] and examination of ≤3 markers.[10,18] Additionally, most previous studies have not examined the relation of exposure acuity on markers.

We sought to study markers that would reflect critical phases in the inflammatory pathway of atherosclerotic plaque development. Up-regulation of serum and plasma CD40L, urinary isoprostanes, and P-selectin are thought to represent the initiation of the atherothrombotic cascade by stimulating cytokines (such as tumor necrosis factor-α, whose effects are mediated in part by TNFR2) in response to damage of the endothelium or vascular smooth muscle.[19,20] P-selectin and ICAM-1 are involved in the rolling phase of leukocyte adhesion and early plaque development.[15,19] MCP-1, a chemokine found in atherosclerotic plaque, attracts monocytes to developing plaque.[21,22] MPO, a leukocyte enzyme, is elevated in culprit lesions of patients with acute coronary syndrome.[20] Urinary isoprostanes indexed to urinary creatinine (isoprostanes) are measures of oxidative stress.[23] The remainder of the markers in our panel (fibrinogen, CRP, and IL-6) are non-specific inflammatory markers found in multiple sites. The current investigation expands upon previous reports by examining a broad panel of circulating biomarkers that we hypothesized reflects the multiple inflammatory pathways activated by smoking. Further, we hypothesized that inflammatory markers would be elevated in a dose-response relation to smoking, and that acute exposure would result in higher inflammatory markers than less proximate exposure. We analyzed the relation of various aspects of smoking behavior to circulating CD40L, CRP, fibrinogen, ICAM-1, IL-6, urinary isoprostanes, MCP-1, MPO, P-selectin, and TNFR2 in the community-based Framingham Heart Study.

Methods

Study sample

We examined participants of the Framingham Heart Study Offspring cohort (largely white of European descent) and the Omni study of ethnic/racial minorities (African Americans, Hispanic Americans and Asian Americans), who attended Exams 7 and 2, respectively (1998–2001). Design and inclusion criteria of the Framingham Heart Study and the Omni Cohort have been described elsewhere.[24–26] The Framingham Study is reviewed by the Boston University Medical Center Institutional Review Board and all participants gave written informed consent.

Baseline measurements and definitions

Smoking status information was obtained by self-report. Current smokers were defined as those who smoked ≥1 cigarette per day regularly during the year preceding the exam. Current smokers were subdivided into 2 categories: those with acute smoke exposure, defined as having smoked ≤6 hours prior to phlebotomy, and those having smoked >6 hours prior to phlebotomy at the index examination. Former smokers were defined as those who denied having smoked regularly for the year preceding the exam, but reported regular smoking more than one year prior to the exam. In both the current and former smokers, assessment of cumulative smoke exposure was measured by calculating pack-years, defined as the number of packs per day multiplied by the number of years smoked. Never smokers were defined as those who reported no regular smoking at or prior to any examination. Chewing tobacco, pipe, cigar smoking, and secondhand smoke exposure were not collected at Offspring examination 7 and therefore were not considered in our analysis.

Laboratory analyses

For analysis, serum, plasma and urine samples stored at −80° C were thawed at room temperature. Samples were assayed between 2001 and 2005 (3 years [isoprostanes] to 8 years [TNFR2] after collection). Measurement reproducibility was good, with intra-assay coefficients of variation (CV) as follows: serum and plasma CD40L 4.7±4.4% and 4.4±3.4%, respectively, serum ICAM-1 3.7±2.4%, serum IL-6 3.1±2.1%, serum MCP-1 3.8±3.3%, serum MPO 3.0±2.5%, plasma P-selectin 3.0±2.2%, and plasma TNFR2 2.2±1.6% were measured using the Molecular Devices VersaMax microplate reader and commercially-available quantitative ELISA kits [Bender MedSystems (CD40L), R&D Systems (ICAM-1, MCP-1, P-selectin, TNFR2), OXIS (MPO), and Cayman (isoprostanes)]. Plasma fibrinogen was measured using the Clauss method (clot time), with Diagnostica Stago Reagents; the intra-assay CV was 2.1%. Serum and plasma CD40L, fibrinogen, ICAM-1, IL-6, isoprostanes, MCP-1, MPO, P-selectin, and TNFR2 were run in duplicate and averaged. Serum CRP was measured on a Dade Behring BN100 nephelometer intra-assay CV 3.2%. Urinary isoprostane concentrations, 8-Epi-$PGF_{2\alpha}$, were indexed to creatinine and had an intra-assay CV 9.1±5.8% ng/mL. Urinary creatinine was measured on an Abbott Spectrum CCX using a technique previously reported.[23]

Statistical analysis

Since the biomarker concentrations were skewed, we logarithmically transformed the markers. In order to have comparable interpretations, we further standardized all biomarkers to have mean 0 and standard deviation 1. Multivariable analysis of covariance was performed for each inflammatory marker with smoking status as the primary exposure of interest and adjusting for 16 covariates: age, sex, cohort (Offspring vs. Omni), body mass index (BMI), waist circumference, systolic blood pressure, diastolic blood pressure, total/HDL cholesterol ratio, triglycerides, fasting glucose, diabetes mellitus, medications (hypertensive, lipid-lowering,

aspirin [>3 days per week], hormone replacement), and prevalent cardiovascular disease. Least-squares means and standard errors were generated for markers according to smoking status (Table 2). A 2-sided $p<0.0045$ ($\alpha=0.05/11$) was considered statistically significant for all analyses to account for multiple testing (11= number of markers analyzed). In secondary analyses, we checked for effect modification by age (>60 vs. ≤60 years old), sex, obesity (BMI <30 vs. ≥30kg/m^2), and cohort (Offspring vs. Omni). We also looked for smoking dose-response relations with inflammatory markers by 2 additional analyses. First, we examined relations between markers and cigarettes per day in current smokers (grouped as 1–9, 10–19, 20–29, ≥30). Second, in ever smokers, we analyzed packs years (grouped as 0–19, 20–39, ≥40) adjusted for former versus current smoking status, and in current smokers for acute versus >6 hours smoking category. SAS version 8.1 was used for statistical analyses.[27]

Results

Participant characteristics and inflammatory markers

We studied the relation of smoking to inflammatory markers in the Framingham Heart Study (n=2944, mean age 60 years, 55% women, 12% ethnic minorities) participants examined from 1998–2001. Clinical characteristics of the cohort stratified by smoking status at examinations 7 and 2 for Offspring and Omni participants, respectively, are shown in Table 1.

Inflammatory markers and smoking status

Adjusting for 16 potential clinical confounders, we observed that marker concentrations differed significantly ($p<0.0045$) across smoking groups (never, former, >6 hours smoking, and acute smoking (≤6 hours from phlebotomy), except for serum CD40L, MPO, and TNFR2 (Figure 1; Table 2). MPO and TNFR2 showed borderline significant differences by smoking category ($p<0.05$ but >0.0045). Former smokers had significantly lower concentrations of plasma CD40L ($p<0.0001$), and higher concentrations of CRP ($p=0.002$), as compared with never smokers. Compared with never smokers, those with exposure >6 hours prior to marker assessment had significantly lower concentrations of plasma CD40L, and higher concentrations CRP, ICAM-1, IL-6, and isoprostanes. Similarly, compared with never smokers, smokers with acute exposure (≤6 hours) had significantly higher concentrations of all markers except serum CD40L, MPO, and TNFR2 compared with never smokers ($p<0.0001$); plasma CD40L concentrations were lower.

Secondary analyses

A subgroup analysis restricted to current smokers was performed to determine if there was a dose-response relation between marker concentrations and number of cigarettes smoked daily. Current smokers were parsed into 4 groups by number of cigarettes smoked per day (mean ±SE): <10 (4.4±0. 2) cigarettes per day, 10–19 (12.3±0.3) cigarettes per day, 20–29 (20.5±0.1) cigarettes per day, and 30 or more cigarettes per day (36.0±0.9). There was a significant trend ($p=0.001$) for increased isoprostanes concentration across increased category of cigarettes smoked per day: the mean±SE isoprostanes concentration (ng/mmol) was 191±12 for <10; 232 ±11 for 10–19; 252±15 for 20–29 and 269±18 for ≥30 cigarettes per day. The trend was not significant for the remaining markers in the panel.

We also examined ever smokers by pack-years of exposure to determine whether there existed a dose-response relation between marker concentrations and cumulative cigarette smoking exposure, adjusting for smoking status (former vs. current) and acuity of exposure (>6 hours vs. acute exposure). Ever smokers were parsed into the following groups according to pack-years of exposure (mean +/− SE): <20 (8.3±0.2), 20–39 (29.7±0.3), and 40 or more pack-years exposure (60.7±0.8). Compared with smokers who had fewer than 20 pack-years of exposure, there was a significant trend towards elevation of CRP, ICAM-1, IL-6, isoprostanes, P-selectin,

and fibrinogen concentrations (Table 3) and lower concentrations of plasma CD40L with increasing pack-years of exposure.

Effect modification

We tested for interactions by BMI <30 vs. ≥30kg/m^2, age ≤60 vs. >60 years, sex (men vs. women), and cohort (Offspring vs. Omni). We observed 2 instances of significant ($p<0.0045$) effect modification (Table 4). First, there was an interaction between obesity and smoking for ICAM-1 concentrations: although obese participants had higher ICAM-1 concentrations than non-obese in 3 smoking categories, among acute smokers, the group with obesity had lower ICAM-1 concentrations than the non-obese group. Second, there was an interaction between age and smoking for CRP: whereas never smokers had similar CRP concentrations for younger and older participants, among former and current smokers, there were higher CRP concentrations in older as compared with younger participants.

Discussion

Our panel of inflammatory markers was significantly elevated in current and former smokers as compared with never smokers. Examining individual markers across smoking groups, CRP concentrations were significantly elevated and plasma CD40L concentrations were significantly lower in former smokers. Current smokers had elevations of several systemic markers reflecting activation of many inflammatory pathways. Additionally, we observed a trend between lower plasma CD40L, and higher CRP, ICAM-1, IL-6, isoprostanes, P-selectin, and fibrinogen concentrations and cumulative tobacco exposure (pack-years) in ever smokers. Taken together, our results are consistent with the hypothesis that several systemic inflammatory pathways are activated by cigarette smoking, an association that is less notable in past smokers.

Individual Markers

It is unclear why we did not find significant elevations in serum CD40L, TNFR2, or MPO; although for the latter two markers the lack of significance was partly due to accounting for multiple testing. We also found a statistically significant inverse relation between smoking status and plasma CD40L, unlike others.[21] Potential explanations for the contrast between our findings with respect to serum and plasma CD40L compared with others may be due to differences in biomarker collection techniques, study sample, covariate adjustment, and smoking status definitions. We are uncertain why plasma CD40L concentrations were higher in never smokers, and smokers with the fewest pack-years exposure, in contrast to the lack of a trend with serum CD40L. Our study is consistent with prior studies demonstrating that soluble CD40L concentrations vary by whether they are measured in serum or in plasma.[28]

Prior literature on serum TNFR2 concentrations in smokers is conflicting. Soluble TNF receptors compete with membrane-bound receptors for binding tumor necrosis factor, thereby acting as inhibitors of TNF activity on tissue, though in some circumstances they can also bind circulating TNF and prolong its activity in the circulation.[29] Mizia-Stec and colleagues demonstrated that in 122 consecutive patients with stable coronary artery disease admitted to the hospital, TNFR2 levels were higher in those who had never smoked and lower in former smokers, which is consistent with our observations.[30] However, this is in contrast to Fernandez-Real and colleagues, who found increased concentrations of soluble TNFR2 in obese smokers, and identified both fat mass and smoking as independent contributors to 4% of the variance of soluble TNFR2.[31] It is also possible that the prolonged storage (eight years at −80°) for TNFR2 may have played a role. We hypothesize that there is a delicate balance between pro- and anti-inflammatory cytokines and receptors which we were unable to resolve in our sample.

Data on MPO is similarly conflicting. Whereas some investigators have shown that there is increased MPO activity in polymorphonuclear leukocytes in smokers (measured by flow cytometry), others have demonstrated no increased peroxidase activity (quantified by the MPXI, a parameter produced by automated blood counters) in neutrophils of smokers as compared with non-smokers.[32,33] We conclude that current evidence is controversial, and our finding that there was no significant elevation in serum in smokers may reflect the distinction between circulating versus tissue concentrations or may reflect differences in MPO assays.

Effect modification

We noted effect modification for CRP by age ≥60 years, and for ICAM-1 by BMI <30kg/m^2. Whereas we adjusted for prevalent clinically diagnosed CVD in our cohort, in our cross-sectional study we were unable to determine the degree to which variation in atherosclerotic burden might have contributed to elevated CRP concentrations in older participants.

Pathophysiology

Animal models have demonstrated that cigarette smoke increases alveolar macrophages and neutrophils in the lung tissue and increases epithelial permeability.[34,35] A recent review examining the effect of acute cigarette exposure on inflammation and oxidative stress in humans concluded that acute smoke exposure was associated with increased neutrophil and macrophage chemotaxis and activation.[36] Interestingly, it demonstrated that there is a suppressive effect of acute smoke exposure on eosinophils and several inflammatory cytokines, including IL-6, TNF-α, interleukin-2, and interferon-γ. It is hypothesized that a balance of pro-inflammatory and anti-inflammatory factors (such as carbon monoxide) exists in cigarette smoke that functions in concert to disturb pulmonary epithelial architecture.[36] Animal studies examining the effects of smoke exposure on blood inflammatory markers have demonstrated a significant increase in MPO,[37] but not in leukotriene B_4 concentrations.[38] Churg et al. exposed mice with knocked-out p55/p75 TNF-α receptors to cigarette smoke and compared them with control mice. Whereas the controls demonstrated increases in gene expression of TNF-α, neutrophil chemoattractant, macrophage inflammatory protein-2, and macrophage chemoattractant protein-1, the knockout mice did not demonstrate any increases in gene expression for those mediators. The authors concluded that TNF-α is central to acute smoke-induced inflammation and resulting connective tissue breakdown.[35]

In humans, autopsy studies have shown that there is a dose-dependent relation between smoking and degree of aortic and coronary atherosclerosis.[39] However, the precise mechanisms by which cigarette smoking promotes atherosclerosis are not known. A recent review of pathobiological mechanisms of smoking and cardiovascular disease appropriately concluded that whereas epidemiological studies have clearly established an important relation between smoking and cardiovascular morbidity and mortality, the precise balance of thrombosis, endothelial dysfunction, and atherogenesis remains to be elucidated.[40]

Strengths and limitations

We recognize that our study has limitations that warrant consideration. First, the cross-sectional design does not allow us to infer causation between cigarette smoking and inflammatory markers; as an observational study we could neither randomize smoking exposure, nor observe pre- versus post smoking cessation. It is possible that elevations may have been caused by other chronic inflammatory states, particularly in smokers with possible co-existing chronic inflammatory conditions. Second, smoke exposure via other tobacco products (cigars, pipes, smokeless chewing tobacco) or second-hand smoke exposure, were not accounted for and may have led to misclassification of overall tobacco exposure. A recent cross-sectional evaluation of 7599 never-smoking adults from the Third National Health and Nutrition Examination Survey found that those with detectable, low-level (range, 0.05 to 0.215 ng/mL) cotinine were

more likely to have elevated fibrinogen and homocysteine, but not WBC or CRP, as compared with subjects without any detectable cotinine.[41] This very interesting finding warrants further investigation with a broader panel of markers to determine what degree of second-hand smoke exposure results in clinically significant elevations of inflammatory markers. Third, the mean cigarette pack-years and mean cigarette number per day is positively related to smoking status (Table 1) such that there was greater exposure in acute smokers vs. >6 hour smokers vs. former smokers. We estimated the mean marker concentrations by pack-year adjusting for smoking status; however, we acknowledge that with an observational study design we cannot completely eliminate confounding of exposure acuity by magnitude of smoking exposure. Fourth, we did not have serum cotinine concentrations available to corroborate self-reported smoking behaviors; we acknowledge that some misclassification of smoking exposure is inevitable. Fifth, there is limited on no data on the stability of several of our markers (ICAM-1, IL-6, MCP-1, MPO, P-selectin, fibrinogen) with long-term storage. It is possible that concentrations may be altered with storage. Sixth, the Framingham cohort is a predominantly white and middle-aged to elderly sample. We attempted to broaden the applicability of our study to other ethnicities and races by including subjects enrolled in the minority Omni study, but we lacked power to examine the relation between smoking exposure and specific ethnic/racial minority subgroup status. Finally, although the trend test between pack-years and marker concentrations was significant for CRP, fibrinogen and ICAM-1, we lacked power to examine threshold effects.

Conclusion

Our data support previous findings that certain biomarkers are increased with cigarette smoke exposure, specifically, CRP, fibrinogen,[42] ICAM-1,[11] IL-6,[9] urinary isoprostanes,[43] and P-selectin.[17] The pattern of marker elevation did not localize to a particular inflammatory pathway thereby confirming the complexity of biochemical events that occur in smokers. The influence of acute cigarette smoke on inflammation may not be completely reversible, as evidenced by the elevated CRP concentrations in the former smokers.

In an era when almost 25% of adolescents and up to 45% of adults in both the European Union and North America continue to smoke, it is essential to identify potential mechanistic pathways that can ultimately be intervened upon to reduce risk of cardiovascular complications in smokers.[4,7,44]

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Abbreviations

BMI	body mass index
CD40L	CD40 ligand
CRP	C-reactive protein
ICAM-1	intercellular adhesion molecule-1
isoprostanes	urinary 8-epi-prostaglandin $F_{2\infty}$ indexed to urinary creatinine
IL-6	interleukin-6
MCP-1	monocyte chemoattractant protein-1

MPO myeloperoxidase

TNFR2 tumor necrosis factor receptor 2

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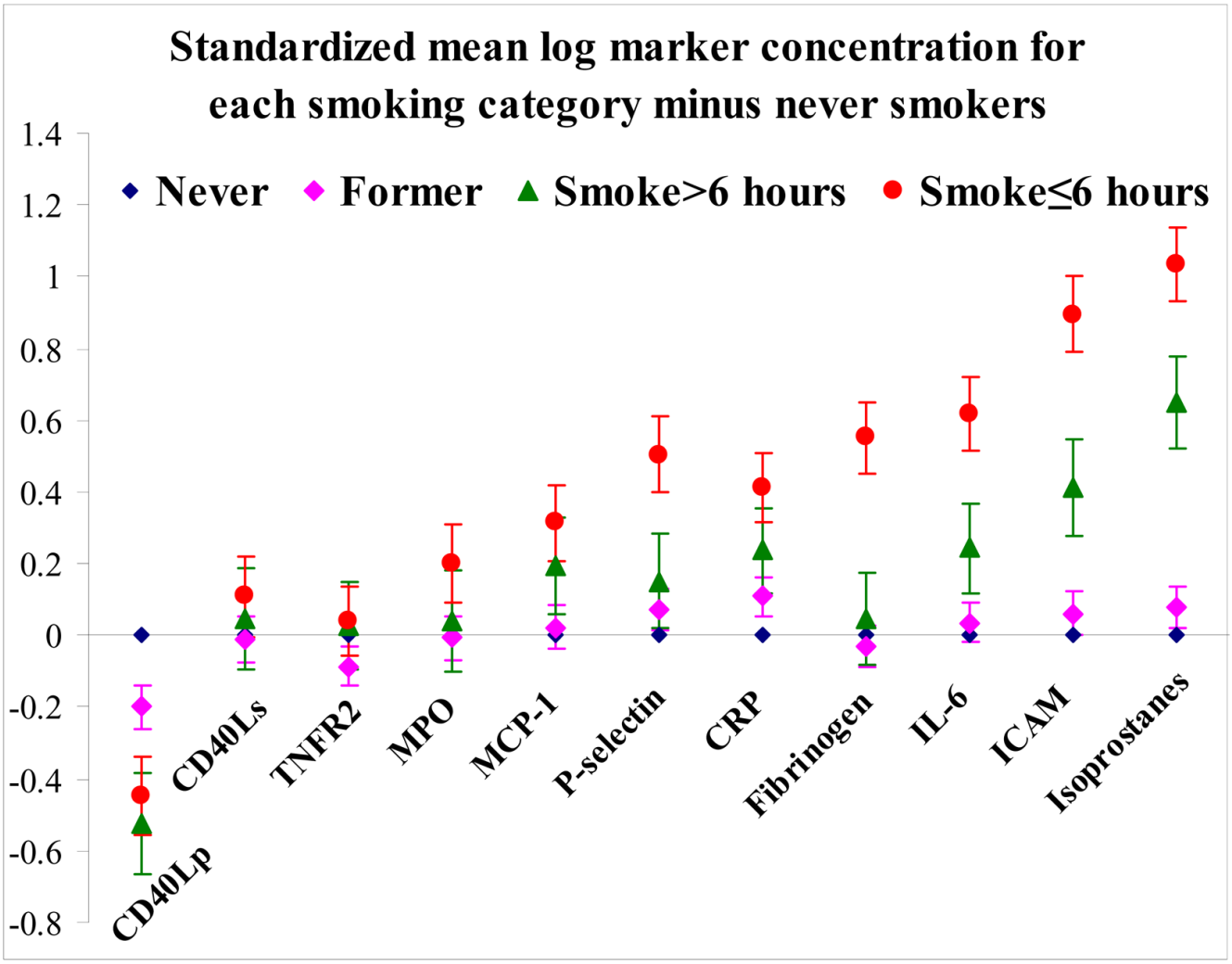

Figure 1.
Estimated mean differences (with standard error bars) for smoking categories versus referent never smokers for standardized log marker concentrations Zero represents no difference between smoking category and never smokers.

Table 1

Clinical characteristics of study sample by smoking status*

	Never smokers n=1149	Former smokers n=1424	Current smokers, Acute Exposure ≤6 >6 hours n= 134	hours n=237
Age, years	60±10	62±9	57±9	57±9
Sex, % female	60	50	56	54
Omni cohort, %	20	7	11	6
Systolic blood pressure, mmHg	128±20	128±18	124±17	123±17
Diastolic blood pressure, mmHg	76±10	74±10	74±10	74±10
Waist circumference, cm	39.1±5.7	39.7±5.4	39.1±6.2	38.2±5.9
Body mass index, kg/m^2	28.1±5.5	28.5±5.3	27.8±5.5	27.2±5.3
Total cholesterol/HDL ratio	4.0±1.4	4.0±1.3	4.0±1.3	4.2±1.5
Triglycerides, mg/dl	135±88	137±85	149±117	139±99
Diabetes, %	12	15	7	11
Fasting glucose, mg/dl	104±30	105±30	103±27	104±29
Hypertension treatment, %	31	37	20	24
Lipid-lowering treatment, %	16	24	15	13
ASA treatment, %	26	35	22	25
Prevalent CVD, %	9	15	11	13
Hormone replacement therapy, %	16	17	19	14
Mean pack-years of cigarettes	-	23±21	32±22	48±23
Current mean # of cigarettes/day	-	-	12±10	20±10

*Characteristics expressed as % (dichotomous) or mean±SD (continuous)

Table 2

Estimated geometric means (95% CI) of inflammatory markers by smoking category

	Never smoker	Former smoker	>6 hours	Acute(≤6 hours)	p-value
CD40L, plasma, ng/mL	1.83 (1.70, 1.96)	1.43 (1.34, 1.52)*	0.96 (0.78, 1.18)*	1.05 (0.90, 1.23)*	<**0.0001**
CD40L, serum, ng/mL	2.28 (2.09, 2.48)	2.24 (2.07, 2.42)	2.45 (1.90, 3.14)	2.67 (2.21, 3.23)	0.36
CRP, mg/L	1.99 (1.88, 2.10)	2.25 (2.14, 2.36)*	2.60 (2.21, 3.06)*	3.17 (2.80, 3.58)*	<**0.0001**
Fibrinogen, mg/dL	370 (367, 374)	368 (365, 372)	374 (363, 384)	411 (402, 420)*	<**0.0001**
ICAM-1, ng/mL	234 (230, 238)	239 (235, 243)	266 (253, 280)*	309 (298, 321)*	<**0.0001**
IL-6, pg/mL	2.72 (2.62, 2.82)	2.79 (2.70, 2.88)	3.23 (2.89, 3.60)*	4.21 (3.88, 4.57)*	<**0.0001**
Isoprostanes, ng/mmol	120 (116, 124)	126 (122, 129)†	177 (161, 194)*	223 (208, 240)*	<**0.0001**
MCP-1, pg/mL	295 (289, 301)	298 (292, 303)	317 (299, 336)†	331 (317, 346)*	<**0.0001**
MPO, ng/mL	39.4 (38.1, 40.7)	39.3 (38.1, 40.4)	40.3 (36.7, 44.4)	44.1 (41.1, 47.4)†	0.03
P-selectin, ng/mL	35.0 (34.3, 35.7)	36.0 (35.3, 36.6)	37.0 (34.9, 39.3)	42.2 (40.3, 44.1)*	<**0.0001**
TNFR2, pg/mL	2022 (1991, 2055)	1969 (1941, 1997)†	2040 (1948, 2135)	2048 (1978, 2120)	0.03

P-value from ANCOVA test of equality among all 4 group means adjusted for covariates listed in statistical methods. P-value from test of specific smoking category versus never smokers:

*significant $p<0.0045$;

†borderline $0.0045 < p < 0.05$

Table 3

Estimated geometric means (95% CI) of marker concentrations by pack year category in ever smokers

	Pack-years			p-value for trend across pack year categories
	<20	**20–39**	**≥40**	
CD40L plasma, ng/mL	1.40 (1.22, 1.60)	1.05 (0.91, 1.22)	0.88 (0.76, 1.01)	<**0.0001**
CD40L serum, ng/mL	2.43 (2.06, 2.86)	2.66 (2.24, 3.16)	2.38 (2.00, 2.82)	0.98
CRP, mg/L	2.22 (2.00, 2.47)	2.83 (2.53, 3.16)	2.76 (2.48, 3.09)	<**0.0001**
Fibrinogen, mg/dL	383 (376, 390)	395 (387, 403)	394 (387, 402)	**0.003**
ICAM-1, ng/mL	246 (238, 254)	266 (257, 275)	263 (254, 271)	<**0.0001**
IL-6, pg/mL	3.05 (2.84, 3.27)	3.51 (3.26, 3.79)	3.65 (3.39, 3.93)	<**0.0001**
Isoprostanes, ng/mL	139 (131, 148)	162 (152, 172)	181 (170, 193)	<**0.0001**
MCP-1, pg/mL	279 (268, 290)	293 (281, 305)	292 (281, 304)	0.02
MPO, ng/mL	37.8 (35.5, 40.2)	36.8 (34.5, 39.4)	38.5 (36.0, 41.1)	0.78
P-selectin, ng/mL	37.7 (36.3, 39.2)	40.0 (38.4, 41.7)	40.8 (39.1, 42.4)	**0.0003**
TNFR2, pg/mL	1921 (1864, 1979)	1978 (1916, 2041)	1975 (1914, 2038)	0.07

[†]Estimated regression coefficients (standard error) for log marker concentrations; the reference group is <20 pack-years, marker concentrations standardized to mean 0, SD 1.

Table 4

Significant interactions: estimated geometric means (95% CI) of inflammatory markers by smoking category and clinical variable

	Smoking Category				Interaction p value*
	Never	**Former**	**>6 hours**	**Acute**	
			CRP (mg/L) Age, ≤60 vs. >60 years		
Age≤60 years	1.89(1.74,2.05)	1.98(1.82,2.16)	2.00(1.63,2.44)	2.61(2.24,3.05)	**0.002**
Age >60 years	1.96(1.78,2.15)	2.44(2.23,2.67)	3.63(2.73,4.84)	3.93(3.13,4.92)	
			ICAM-1 (ng/mL) BMI, kg/m^2		
BMI<30kg/m^2	220(215,226)	225(220,231)	249(235,265)	306(292,320)	**0.0003**
BMI≥30	230(222,239)	233(225,241)	264(240,290)	262(242,282)	

We display only statistically significant interactions (p<0.0045, to account for multiple testing). Analyses were conducted on log markers.

*p-value from ANCOVA test of interaction between smoking categories and specified clinical variable adjusted for covariates listed in methods.